A.M.A. Archives of
OPHTHALMOLOGY

OCTOBER 1956

VOLUME 56 NUMBER 4

Retrolental Fibroplasia
V. Everett Kinsey

General Manifestations and Symptoms of Collagen Diseases
Frank H. Constantine

Pathology of Diffuse Collagen Diseases
Lorenz E. Zimmerman

Ocular Manifestations of Collagen Diseases
A. Edward Maumenee

Differential Diagnosis of Collagen Diseases
Jack S. Guyton

Evolution of the Collagen Diseases
René Contardo

The Therapy of Collagen Diseases
Irving H. Leopold

Physiological Chemistry of the Eye
Zacharias Dische

Complete Contents on First Inside Page

AMERICAN MEDICAL ASSOCIATION Publication

a scientific corner

FOR INFORMAL DISCUSSION OF YOUR OPTICAL PROBLEMS

The House of Vision Inc.

CHICAGO

EVANSTON	HIGHLAND PARK	OAK PARK	AURORA
DES MOINES	MASON CITY	MILWAUKEE	MINNEAPOLIS
MUSKEGON	SIOUX CITY	AMES	DAVENPORT

WHAT CATARACT LENS IS BEST???

Different types of cataract bifocals produce markedly different effects in weight, appearance and optical performance. Studying the various types of cataract bifocals most commonly used, the following is our opinion:—

In many cases the ground lenticular is to be recommended. Where you must use a large lens, one with large amounts of decentration, or one with an eccentric shape, it is the only practical form to keep weight at a minimum. However, this bifocal form accentuates the strength of the lens because of the reflections of the intersection of lenticular and plano fields. Further, a fused segment always causes *some* chromatic aberration and distortion.

Fused lenticulars do improve the appearance somewhat—they save about 3 to 3.5 diopters in the distance portion of the lens.

Where lens size can be controlled, where there is a small amount of decentration and a conservative shape, our preference for the best optical design in a cataract case is the solid one-piece bifocal lens with the segment on the convex side of the lens. This permits the cylinder to be ground on the minus side. With eye size kept to a minimum and the lens properly ground to a knife edge, we believe that the best optical performance and best appearance are obtained.

"if it's a lens problem, let's look at it together"

THE HOUSE OF VISION—MAKERS OF PRESCRIPTION GLASSES FOR THE MEDICAL PROFESSION—WILL BE HAPPY TO FILL *YOUR* PRESCRIPTIONS.

TABLE OF CONTENTS

VOLUME 56 OCTOBER 1956 NUMBER 4

ORIGINAL ARTICLES

PAGE

Retrolental Fibroplasia
V. Everett Kinsey, Ph.D., Detroit, with the Assistance of June Twomey Jacobus, B.A., Detroit, and F. M. Hemphill, Ph.D., Ann Arbor 481

SYMPOSIUM ON COLLAGEN DISEASES

General Manifestations and Symptoms of Collagen Diseases
Frank H. Constantine, M.D., New York 544

Pathology of Diffuse Collagen Diseases
Lorenz E. Zimmerman, M.D., Washington, D. C. 548

Ocular Manifestations of Collagen Diseases
A. Edward Maumenee, M.D., Baltimore 557

Differential Diagnosis of Collagen Diseases
Jack S. Guyton, M.D., Detroit 563

Evolution of the Collagen Diseases
René Contardo, M.D., Santiago, Chile 568

The Therapy of Collagen Diseases
Irving H. Leopold, M.D., Philadelphia 577

ANNUAL REVIEWS

Physiological Chemistry of the Eye
Zacharias Dische, M.D., New York 587

REGULAR DEPARTMENTS

News and Comment 635
Books 638

A. M. A.
ARCHIVES OF OPHTHALMOLOGY

VOLUME 56 OCTOBER 1956 NUMBER 4

COPYRIGHT, 1956, BY THE AMERICAN MEDICAL ASSOCIATION

Published monthly by the AMERICAN MEDICAL ASSOCIATION. Editorial and Circulation Offices: 535 North Dearborn Street, Chicago 10, Illinois. Publication Office: Thompson Lane, Box 539, Nashville 1, Tennessee. Change of Address: Notice to the circulation office should state whether or not change is permanent and should include the old address. Six weeks' notice is required to effect a change of address. Second-class mail privileges authorized at Nashville, Tenn., Aug. 6, 1956.

Continuing the Publication
FOUNDED BY HERMAN KNAPP, 1869

EDITORIAL BOARD

FRANCIS HEED ADLER, Chief Editor
313 South Seventeenth Street, Philadelphia 3

W. L. BENEDICT, Rochester, Minn.
DAVID G. COGAN, Boston
JOHN H. DUNNINGTON, New York
MICHAEL J. HOGAN, San Francisco
WILLIAM F. HUGHES Jr., Chicago
KENNETH C. SWAN, Portland, Ore.
WILLIAM ZENTMAYER, Philadelphia

ASSOCIATES:

Alson E. Braley
F. Phinizy Calhoun Jr.
Zacharias Dische
W. Morton Grant
Trygve Gundersen
Joseph S. Haas
Albert N. Lemoine Jr.
John M. McLean
Paul W. Miles
Marshall M. Parks
Frank Payne
Henry P. Wagener

AUSTIN SMITH, Editor, A. M. A. Scientific Publications
GILBERT S. COOPER, Managing Editor, Specialty Journals

AMERICAN MEDICAL ASSOCIATION SCIENTIFIC PUBLICATIONS

A. M. A. Specialty Journals

Monthly

A. M. A. Archives of Internal Medicine. Paul S. Rhoads, M.D., Chief Editor, American Medical Association, 535 N. Dearborn St., Chicago 10. Price $10.00; Canada, $10.50; Foreign, $11.50; individual copy, $1.00.*

A. M. A. Archives of Dermatology. Herbert Rattner, M.D., Chief Editor, 104 S. Michigan Ave., Chicago. Price, $12.00; Canada, $12.50; Foreign, $13.50; individual copy, $1.25.†

A. M. A. Archives of Ophthalmology. Francis Heed Adler, M.D., Chief Editor, 313 S. 17th St., Philadelphia 5. Price, $12.00; Canada, $12.50; Foreign, $13.50; individual copy, $1.25.†

A. M. A. Archives of Surgery. Waltman Walters, M.D., Chief Editor, American Medical Association, 535 N. Dearborn St., Chicago 10. Price, $14.00; Canada, $14.50; Foreign, $15.50; individual copy, $1.25.‡

A. M. A. Journal of Diseases of Children. Robert B. Lawson, M.D., Chief Editor, 1000 N. W. 17th St., Miami 36, Fla. Price, $12.00; Canada, $12.50; Foreign, $13.50; individual copy, $1.25.†

A. M. A. Archives of Pathology. Paul R. Cannon, M.D., Chief Editor, Department of Pathology, University of Chicago, The School of Medicine, 950 E. 59th St., Chicago 37. Price, $10.00; Canada, $10.50; Foreign, $11.50; individual copy, $1.00.*

A. M. A. Archives of Neurology and Psychiatry. Harold G. Wolff, M.D., Chief Editor, Neurology, 525 E. 68th St., New York 21; Roy R. Grinker Sr., M.D., Chief Editor, Psychiatry, Institute for Psychosomatic and Psychiatric Research, 29th St. and Ellis Ave., Chicago 16. Price, $14.00; Canada, $14.50; Foreign, $15.50; individual copy, $1.50.‡

A. M. A. Archives of Otolaryngology. George M. Coates, M.D., Chief Editor, 1721 Pine St., Philadelphia 3. Price, $14.00; Canada, $14.50; Foreign, $15.50; individual copy, $1.50.‡

A. M. A. Archives of Industrial Health. Prof. Philip Drinker, Chief Editor, Dept. of Industrial Hygiene, Harvard University School of Public Health, 55 Shattuck St., Boston 15. Price, $10.00; Canada, $10.50; Foreign, $11.50; individual copy, $1.00.*

* Price to students, interns, and residents: $6.00 in U. S. & possessions.

† Price to students, interns, and residents: $7.00 in U. S. & possessions.

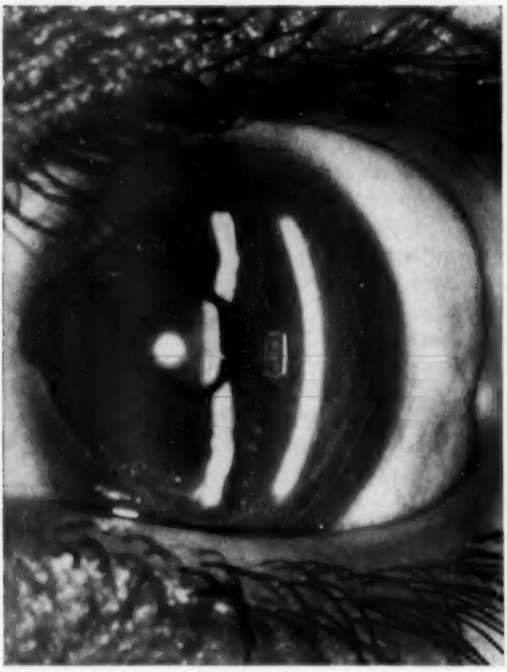
‡ Price to students, interns, and residents: $8.00 in U. S. & possessions.

The price for individual copies applies only to this and the previous calendar year. Back issues older than two years are available through Walter J. Johnson, Inc., 111 Fifth Avenue, New York 3. Future reprints of back issues will be available through Johnson Reprint Corporation, 111 Fifth Avenue, New York 3.

The Journal of the American Medical Association. Weekly. Annual Subscription Price: Domestic, $15.00 Canadian, $17.00. Foreign, $21.50. Price to students, interns, and residents: $9.00 in U. S. & possessions.

Quarterly Cumulative Index Medicus. Issued Twice a Year. Subscription Price, Calendar Year, $25.00.

Checks, money orders, and drafts should be made payable to the American Medical Association, 535 North Dearborn Street, Chicago 10.



Topically effective sight safeguards

STERILE OPHTHALMIC SUSPENSIONS
AND OPHTHALMIC OINTMENT

HYDROCORTONE®
ACETATE

(HYDROCORTISONE ACETATE, MERCK)

Topically administered hydrocortisone has proved to be "superior (to cortisone) in ...: contact dermatitis of the lids; vernal conjunctivitis; sclerosing keratitis; superficial punctate keratitis; recurrent corneal erosions, and diffuse episcleritis..." "Most other lid and anterior segment diseases responded equally well either to cortisone or hydrocortisone..."

Supplied: Sterile Ophthalmic Suspensions of HYDROCORTONE Acetate—0.5 and 2.5 per cent; 5-cc. dropper bottles. Ophthalmic Ointment of HYDROCORTONE Acetate—1.5 per cent; 3.5-Gm. tubes.

Reference: Hogan, M. J., Thygeson, P. and Kimuras, J., Arch. Ophth. 53:165, (Feb.) 1955.

MSD

MERCK SHARP & DOHME
DIVISION OF MERCK & CO., INC., PHILADELPHIA 1, PA.

TO OUR SUBSCRIBERS AND CONTRIBUTORS

SUBSCRIBERS and contributors to this journal have undoubtedly noticed some deviation from our usual publishing quality in the July and August and September issues. In particular, authors have had to correct very bad proofs in less than adequate time for careful reading. Subscribers must have detected an unusual number of typographical errors which were not corrected from the galleys. A most disturbing fact was the late mailing of these issues. Many of our authors and readers were quick to realize that such a departure from our usual standards could be due only to abnormal conditions, and we feel that these conditions need to be explained.

On April 23, 1956, the American Medical Association concluded negotiations for a contract with the Baird-Ward Printing Company, Inc., Nashville, Tenn., to print the American Medical Association Specialty Journals. Under the terms of the contract, the Baird-Ward Printing Company, Inc., was to begin with the July issues.

A transfer of a printing operation requires that many details be considered and many adjustments be made. A contract as large as this involved expanding a work force with skilled operators, not always readily available; installing additional machinery and type equipment not obtainable on short notice, and scheduling nine journals so as to coordinate their production with other jobs in the plant. Editorially, it meant increased pressure upon editors to meet an advanced schedule, the development of a common interpretation of editing procedures between typesetter and manuscript editor, and the working out of communications between the Editorial Office, printing plant, authors, and readers.

All this was a Herculean task in view of the short period of time allowed for the transfer. Obviously, substandard results could not be avoided. While a contributor or a reader is justly concerned with his individual article or copy, it is hoped that the realization of the many ramifications of the transfer will soften criticism of the initial effort, performed under an entirely new set of production conditions.

Many of the early problems have been eliminated. Others are being solved daily. The efforts of the editorial and manufacturing staffs are directed toward an improvement over what our readers have been offered in the past. Publication dates will be improved, and plans are progressing to make our subject contribution more essential to the profession.

We trust that a better understanding of the circumstances will enlist your patient indulgence in our efforts to return to normal publishing conditions and to keep a promise of a better product.

MANAGING EDITOR

No "before-and-after" demonstration amazes like that of the old flint Kryptok and incomparable Orthogon "D"! You don't need a "practiced" eye to see instantly the clear, sharp, color-free imagery of Orthogon "D"—and its far greater reading range. Orthogon "D" Nokrome combination of crown glass and barium glass segment focuses rays of all colors at a given point. Which would YOU wear?

They Look Alike... But What a Difference!

IN ENGINEERING

IN PERFORMANCE

ORTHOGON "D"

"INVISIBLE" BIFOCAL

BAUSCH & LOMB

TEN

...MILLION, THANKS...

YES, the *total* has changed . . . Browline® frames have passed the eleven million mark, thanks to their still increasing preference.

The *reasons* for preferring Browlines have not changed—their advantages in patient approval of their styling and comfort . . . ease in fitting . . . interchangeability . . . readily available styles, colors and sizes—in depth . . . dependable quality . . . plus the fact they're 'SHURON.'

In your choice of lenses, cases, and machinery, too, "the swing is to Shuron." Your 1955 orders exceeded those of 1954 by more than 20% . . . and are even higher this year.

We appreciate your preference . . . a preference that makes Shuron the world's largest organization devoted exclusively to serving the ophthalmic profession.

SHURON OPTICAL COMPANY, INC. SHURON® **Geneva, N.Y. Rochester, N.Y.**

The Continental

Cat. No. 67

A Suave, Lightweight 1/10 12K Gold-Filled for Men

DETAIL

Eyes	Bridges
42	20, 22, 24
44	20, 22, 24
46	20, 22, 24
48	20, 22, 24

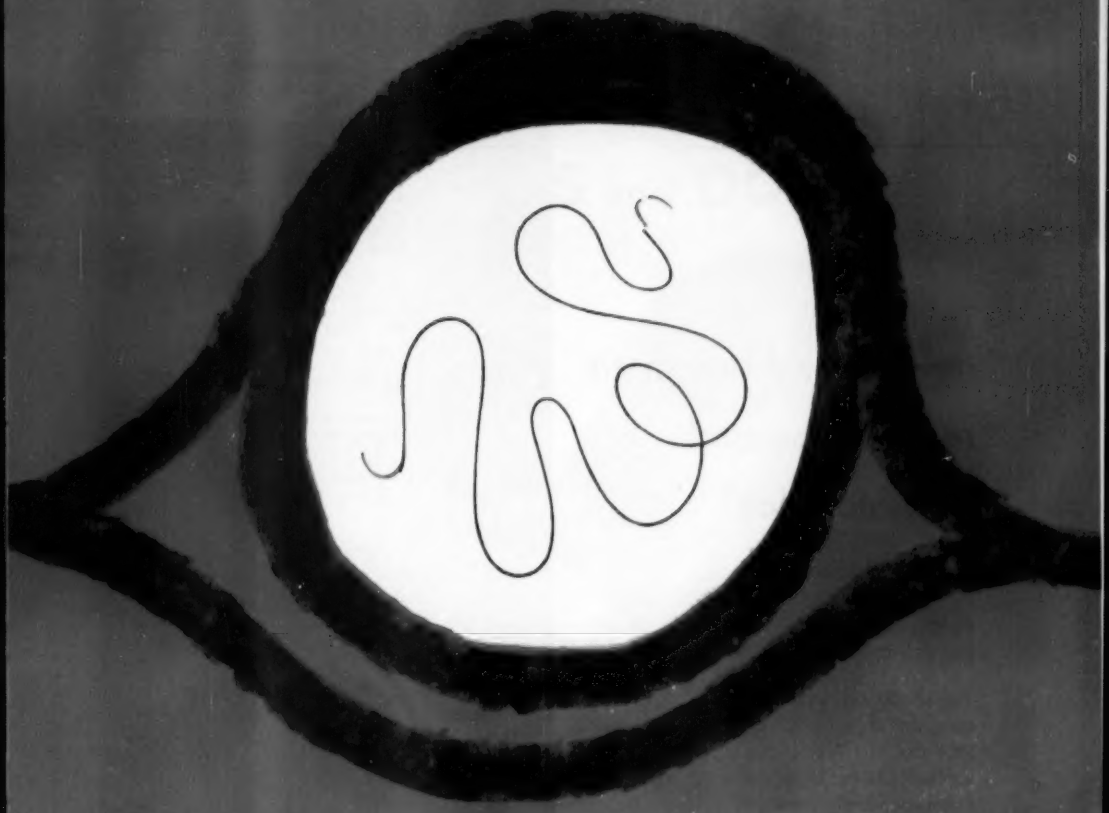
COLORS
Mello and White

TEMPLES—BUTT JOINT
1/10 12 K Gold-Filled.

No. 26 Comfort Cable
No. 29 Skull, Crystal Tips

Many men enjoy the feeling that comes with wearing fine gold eyewear. Others prefer it because it's easy to fit properly, lightweight and comfortable. For both, the new Continental frame is a natural choice. It is clean-cut and masculine. The on-plane bridge is a graceful height and its simplicity is relieved just enough by two engraved horizontal lines. A single line of similar engraving follows the contour of the front and there's an added decorative touch where ends turn back to join the temples. It's good taste in the Ivy League tradition. And, of course, the Continental frame boasts Barreloc which keeps the lenses it holds secure.

Continental INDIANAPOLIS



new needle sutures

for scleral resection and dacryocystorhinostomy

double-armed ophthalmic needle-sutures with special MICRO-POINT® needles designed in consultation with leading ophthalmologists for use in particularly delicate procedures.

ETHICON®

new corneal anesthetic

rapid action and

short duration*

virtually **nonsensitizing**
no cases reported of dermatitis occurring in patient's eye or on hands of physician.

relatively **nonirritating**
does not sting or burn when dropped into the eye — changes in the corneal epithelium or hyperemia of the conjunctiva rarely occur.

Average dose: *for tonometry,* 2 drops. *For minor surgical procedures,* 2 drops instilled 3 times at 90 second intervals.

Supplied: sterile normal saline solution with 0.02% Butoben as preservative, and containing 0.4% Dorsacaine Hydrochloride (benoxinate hydrochloride). In ½ oz. plastic squeeze bottle with dropper tip which delivers uniform drops (approximately 45 drops per cc.).

*Schlegel, H. E., Jr., and Swan, K. C.: A.M.A. Arch. Ophth. *51*:663 (May) 1954.

DORSACAINE®

BENOXINATE HYDROCHLORIDE

SMITH-DORSEY • Lincoln, Nebraska • A Division of The Wander Company

A *Titmus* "Hit" with Custom Fit for LITTLE GUYS and DOLLS

JAMESTOWN, JR.
for Little Guys

Not a "cut-down" man's design, but a rugged-built frame for rough-and-tumble "cowboys". Reverse tear drop pads and contoured bridge provide custom fit for young faces.

COLORS: Demi-Amber Tutone; Demi-Blonde Tutone; Flesh Solid-tone; Cordovan Clear Bridge.

SIZES:
34/18-20-22 38/18-20-22
36/18-20-22 40/18-20-22

Riding Bow Temple Size:
5¼, 5½, 5¾, 6, 6¼"
Taperlite Skull: 5, 5¼"

Twinkle
for Little Dolls

Dainty design for active dolls, quality constructed for comfortable fit.

COLORS: Clear Bridge — Red Magic, Blue Magic, Red Patch, Blue Patch; Solid Bridge Tutone— Red Magic, Blue Magic, Spangle.

SIZES:
34/16-18-20 38/18-20-22
36/16-18-20-22 40/18-20-22

Riding Bow Temple Size:
5¼, 5½, 5¾, 6, 6¼"
Taperlite Skull: 5, 5¼"

TWINKLE DELUXE

Decorated with a beautiful ribbon bow trim in 1/10 12K gold filled.

Titmus OPTICAL COMPANY, INC.
PETERSBURG, VIRGINIA

MANUFACTURERS OF OPHTHALMIC LENSES • FRAMES • SUN GLASSES

Master Styling Room Ensemble

OPHTHALMIC EQUIPMENT AND FURNITURE

● Today's eye doctor must satisfy the growing demand of his patients for fashion, style and service. And at the same time, in the interest of more successful operation, he must increase his own efficiency.

After several years of research and study under actual operating conditions, PROFESSIONAL SUITE presents this matched line of equipment and furniture – *specifically designed and engineered for the modern styling and dispensing rooms of modern doctors . . .* sold in matched ensembles for large, medium and smaller offices. Individual pieces may also be purchased.

P. O. BOX 65
MINNEAPOLIS, MINN.

For further information on color, wood selection and price, discuss PROFESSIONAL SUITE with your supplier or send for this illustrated catalog.

Professional Suite, P. O. Box 65, Minneapolis, Minn.

I'd like to take a look at your PROFESSIONAL SUITE catalog *(no obligation, of course).* Dept. No. A-106

NAME_____

ADDRESS_____

CITY_____ ZONE____ STATE_____

external
ocular
infections...
usually respond rapidly to
topical therapy with one of the
Pfizer broad-spectrum antibiotics,
Tetracyn or Terramycin; Cortril,
an anti-inflammatory hormone,
provides another powerful
therapeutic agent
for ophthalmic use.
Tetracyn®
OPHTHALMIC OINTMENT
5 mg. tetracycline Gm. in ⅛ oz. tubes
Terramycin®
OPHTHALMIC OINTMENT WITH POLYMYXIN B SULFATE
5 mg. of oxytetracycline and 10,000 units of
polymyxin B sulfate Gm. in ⅛ oz. tubes
OPHTHALMIC (FOR SOLUTION)
25 mg. oxytetracycline in 5 cc. dropper vials
Cortril®
OPHTHALMIC OINTMENT
hydrocortisone acetate 5 mg. Gm. in ⅛ oz. tubes
Terra-Cortril®
OPHTHALMIC SUSPENSION
5 mg. oxytetracycline and 10 mg. hydrocortisone
acetate cc. in 5 cc. dropper bottles
Pfizer
PFIZER LABORATORIES, Brooklyn 6, N. Y.
Division, Chas. Pfizer & Co., Inc.

WIES Chalazion Forceps, with flattened top, to allow introduction deep into the fornix. Transverse serrations prevent slippage.
Stainless Steel $11.00 each

NEW
CHALAZION FORCEPS
BY LAWTON

The LAWTON Company
485 FOURTH AVENUE NEW YORK 16, N. Y.

LORDAN Chalazion Forceps, with pointed jaw, to facilitate access to the smaller types of chalazia either at the inner or external canthus.
Stainless Steel $10.50 each

Available Only Through Authorized Surgical Supply Dealers

HEART DISEASE

an indiscriminate killer, touching the young or old

The "well-informed" patient can help you, by helping himself. For reliable information on this subject, have your patients read:

HEALTHY HEARTS

A collection of Hygeia articles: The War Against Heart Diseases Still Rages, Hearts in the Breaking, Preventing Heart Attacks, Heart Diseases of Middle Life.
20 pages, 20 cents

STROKE

In relation to overweight, hardening of the arteries, high blood pressure, blood clots, diabetes.
by William W. Bolton
8 pages, 15 cents

HEART ATTACK

Covering types of attacks, symptoms, relief and heart diseases.
by Walter Modell
12 pages, 15 cents

A KITCHEN FOR THE "TAKE-IT-EASY" COOK

Designed for the cardiac housewife, but the work-saving ideas can be applied in any kitchen. Illustrated.
by Anna May Wilson
16 pages, 20 cents

WRITE TO:

BUREAU OF HEALTH EDUCATION
AMERICAN MEDICAL ASSOCIATION
535 NORTH DEARBORN · CHICAGO 10

storz Surgical Instruments

Improved

Hot Air Sterilizer •

***To Prevent Corrosion of Sharp* Eye Instruments**

E-7070

Sterilizer, Hot Air: features dry heat, constant temperature, forced circulation for even distribution of heat, well insulated for economy and room comfort, fully automatic.

Hot air sterilization eliminates the corrosive damage to sharp, delicate eye instruments so frequent in boiling, autoclaving and some cold sterilizing agents. High temperature is well below point at which temper of sharp instruments would be affected.

Hot air sterilization eliminates bothersome refilling and cleaning of water sterilizer and portable autoclave.

Price: $247.50

Special Features

- Does not draw in outside air.
- Circulation of air in sterilizer chamber insures constant temperature in all areas.
- Good insulation reduces current consumption.
- Even preheating and cooling prevents overheating in any area.
- Fully automatic; requires no attendance after starting, and no special wiring or connections.

Outside dimensions are 20" long, 12" wide, and 15" high. Each of the three trays measures 11" long, 5" wide and 1¼" deep.

Total useful capacity 11" x 6¾" x 7¼".

Weight 40 lbs.

Fully Automatic Controls include INTERNAL TIMER, VISUAL THERMOMETER and INDICATOR LIGHT.

Simplified Operation

After instruments are placed in the sterilizer, the switch at the right rear is moved forward. This includes the time switch in the circuit. The time clock is then set for the required sterilization and preheating time. The preheating time is 20 minutes and normally sterilization time is 30 minutes, a total of 50 minutes. The temperature control knob is set at between 180° and 200° centigrade as desired. Then turn on control switch for operation, which is above the red light on the front. The red light indicates that the sterilizer is on. Thermometer on top of sterilizer can be checked to determine if inside temperature is correct.

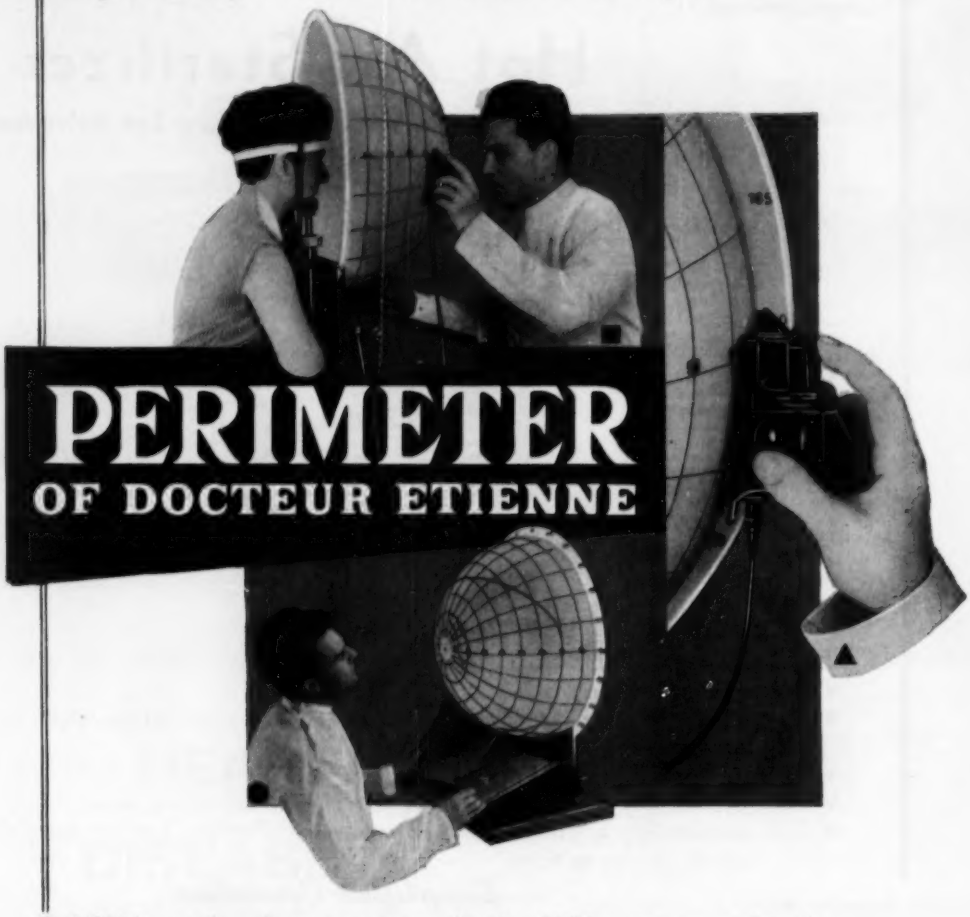
For uninterrupted operation of the sterilizer the time clock switch at the right rear should be pushed back. This removes the time clock from the circuit.

Alternate current (AC) only, 110 Volt, 50-60 Cycle.

Storz Instrument Company

4570 Audubon Ave.
St. Louis 10, Mo.

For a quick, easy and acurate **VISUAL FIELD** *testing*



PERIMETER
OF DOCTEUR ETIENNE

■ BOWL made of transparent plastic. White screen painted on the inside ; meridians and parallels engraved on the outside. Illumination uniform and ajustable.

▲ Ever-clear TEST OBJECT projected from the outside, the position of which is easy to mark.

● During the testing, the limits of the visual field are designed straight on the bowl. After examination, they are recorded on special tracing paper CHARTS. Scotomas can be recorded apart, directly traced on the bowl, with the accuracy of a CAMPIMETER.

GAMBS
4 RUE PRÉSIDENT CARNOT
LYON
FRANCE

And, for the **U.S.A.**
ANTON HEILMAN
220, MAIN STEET
HEMPSTEAD, NY

MODERN D-25

More expensive...*and worth it!*

Threshold-Welded . . . Akro seg glass . . . thin, gray, non-reflecting angled segment top . . . corrected curves, of course . . .

by MODERN OPTICS *Incorporated*

SAN FRANCISCO HOUSTON CLEVELAND

One kid tells another

what little frames are made of...

they're made of smooth, rugged zyl in ice cream colors and warm browns, bright reds and pretty blues... they have light as a feather bridges and temples that can't cut or press... they're better to see with because they stay **on** when you play games or ride a bike...

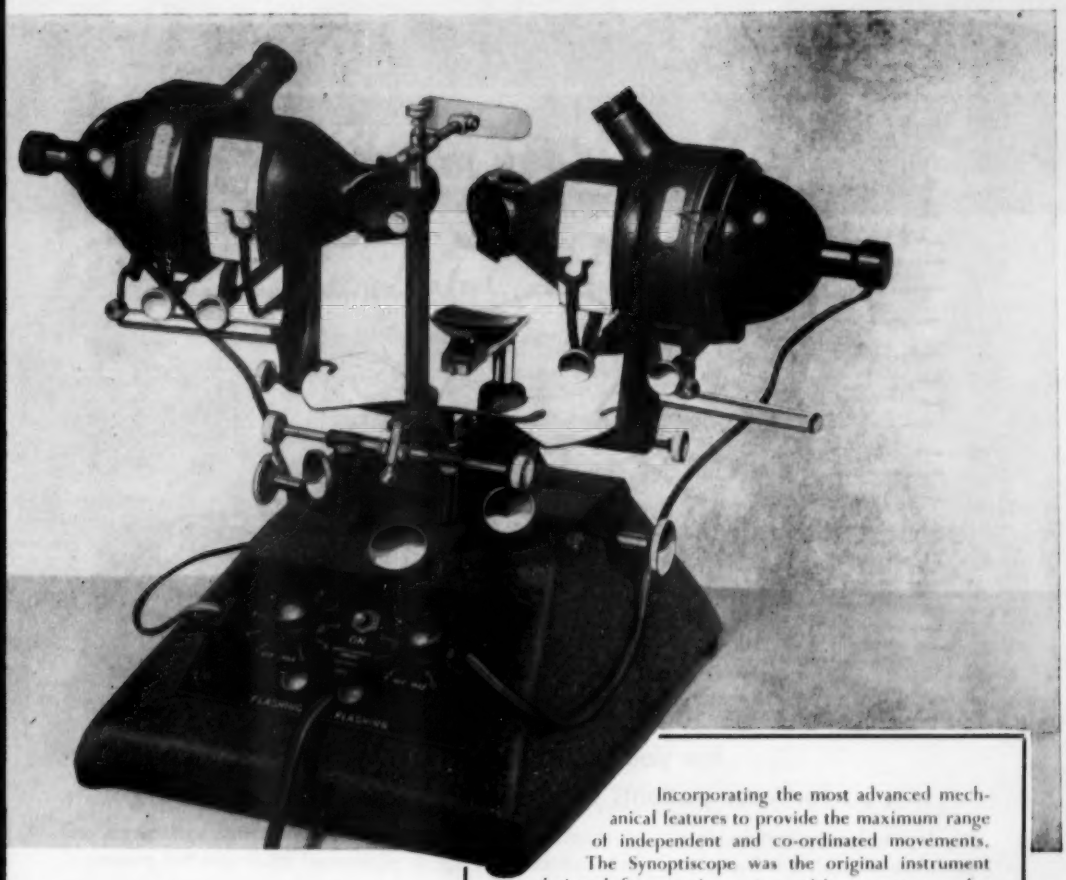
and they're made that way by KONO

the

Smart Way

to help young eyes

KONO



THE SYNOPTISCOPE by CURRY & PAXTON

INCORPORATED

230 PARK AVENUE · NEW YORK 17, N.Y.

Manufacturer's Representatives in the U.S.A.

Service & Assembly Plant:
866 Willis Avenue, Albertson, Long Island, N.Y.
Complete service, repair and parts department

Incorporating the most advanced mechanical features to provide the maximum range of independent and co-ordinated movements. The Synoptiscope was the original instrument designed for carrying out precision movements for Orthoptic training.

MAIN FEATURES

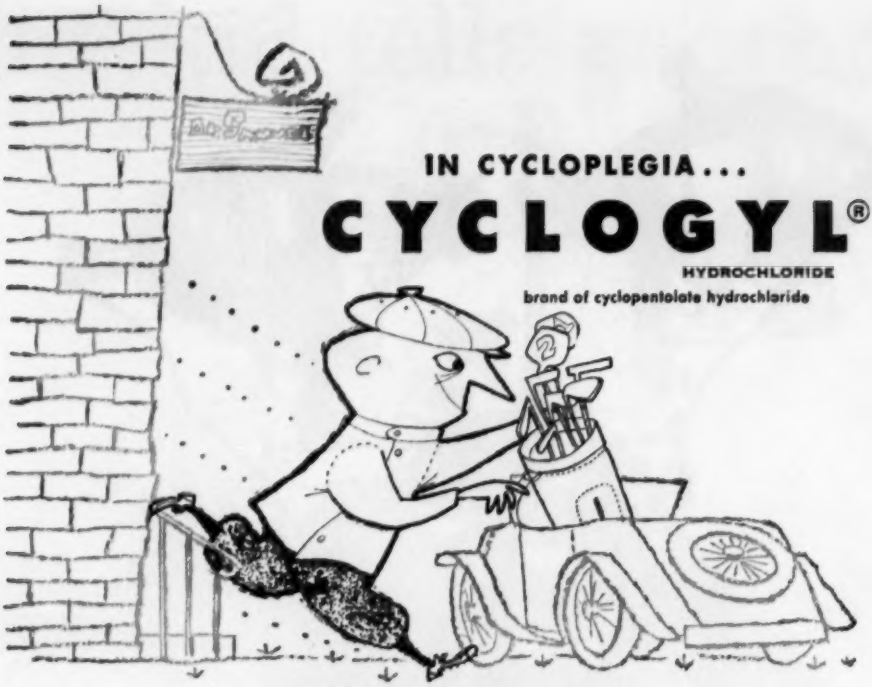
1 **New wide field** eye pieces and larger mirrors, brighter illumination with high intensity gas filled bulbs. 2. **Accurate measurement** of horizontal ductions, vertical ductions and cyclo ductions under the stimulus of Stereoscopic vision or otherwise. 3. **Precise, controlled and unrestricted** exercise of the external ocular motor muscles. *(This function is of especial value where the correcting lenses are not readily accepted by the patient, or where it is required to build up a duction reserve).* 4. **Relaxation exercises.** 5. **Treatment of squint,** including: development of simultaneous macular preception; development of fusion; development of stereoscopic vision and fusional reserve; co-ordination of hand and eye; co-ordinating eye movements.

SUPPLEMENTARY FEATURE
Streak slides for the Bielschowsky after image test.

Other Ophthalmic Instruments manufactured by Curry & Paxton of England include:

SLIT LAMPS · OPHTHALMOSCOPES · THE FOVEOSCOPE · STREAK RETINOSCOPES · MADDOX WING TEST · MADDOX HAND FRAME DIPLOSCOPE · CHEIROSCOPE · TRIAL FRAMES · TRIAL CASES ORTHOPTIC APPARATUS

STRIBUTORS: *Iago:* House of Vision *Los Angeles:* Spratt Optical Superior Optical *San Francisco:* Parsons Laboratories *Pittsburgh:* Doig Optical



IN CYCLOPLEGIA . . .

CYCLOGYL®

HYDROCHLORIDE

brand of cyclopentolate hydrochloride

SAVES TIME

. . . for you . . . with rapid onset of action

. . . for your patients . . . with rapid recovery

"Cyclopentolate hydrochloride . . . produces a rapid [30 to 60 minutes], intense cycloplegia and mydriasis of moderate duration . . . and is effective in highly pigmented irises and for persons of all ages."

New and Nonofficial Remedies, Philadelphia, J. B. Lippincott Company, 1954, p. 189.

Cyclogyl has shown outstanding qualities in 1035 cases.[1]
(Incorporates work of 11 previous investigators)

Compound	Dosage and Administration (drops) *	Diopters of Residual Accommodation	Spontaneous Recovery (in hr.)
Cyclogyl 0.5%	2	1.10	24†
Homatropine 2% or 4%	2	2.00	48

*Successive drops at five-minute intervals.

†Recovery time reduced to six hours after one-percent pilocarpine.

1. Gordon, D. M., and Ehrenberg, M. H.: Am. J. Ophth. 38:831 (Dec.) 1954.

"the chief handicap of maximum cycloplegia, namely its incapacitating effect, is minimized . . ."

Gasteı, B. C.: A.M.A. Arch. Ophth. 51:467 (Apr.) 1954.

Cyclogyl, "by virtue of its brevity, rapidity of action, and consistent depth of cycloplegia, will be [a drug of choice] for routine refraction . . ."

Ehrlich, L. H.: New York J. Med. 53:3015 (Dec. 15) 1953.

Dosage:

Cycloplegia – One drop 1.0%, in each eye (or two drops of 0.5%) produces maximal cycloplegia in 30-60 minutes. Recovery time is normally 4-12 hours spontaneously. Pilocarpine reduces recovery time. In deeply pigmented eyes, 2 drops of 1.0% in each eye, instilled 10 minutes apart, achieves satisfactory cycloplegia in 98% of cases.

Mydriasis – One or two drops of 0.5% in each eye.

Samples and Literature on request.

Supplied:

Sterile ophthalmic solution in Gifford's Buffer (pH 6.0)
0.5% Solution – 15 cc Dropper Bottles
1.0% Solution – 15 cc Dropper Bottles
2 cc Dropper Bottles

Schieffelin & Co Since 1794

Pharmaceutical and Research Laboratories
New York 3, N. Y.

In Canada: William Sofin Ltd.
Montreal 25, Quebec.

A.M.A. Archives OF OPHTHALMOLOGY

Retrolental Fibroplasia

Cooperative Study of Retrolental Fibroplasia and the Use of Oxygen

V. EVERETT KINSEY, Ph.D.
Detroit
With the Assistance of June Twomey Jacobus, B.A., Detroit,
and F. M. Hemphill, Ph.D., Ann Arbor

Table of Contents

	Page
Introduction	482
Purpose	483
Organization	483
Design	484
Protocol	486
Population	488
Mortality in the Routine-and Curtailed-Oxygen Groups	489
Incidence of Retrolental Fibroplasia Routine-and Curtailed-Oxygen Groups	
Active Stages of RLF	490
Cicatricial Grades of RLF	492
Comment	493
Multiplicity of Birth	494
Health Status	495
Duration in Oxygen	496
Concentration of Oxygen	503
Gestational Age	506
Birth Weight	508
Sex	511
Race	512
Type of Incubator	512
Miscellaneous Factors	513
Severity of Retrolental Fibroplasia	514
Spontaneous Regression of Retrolental Fibroplasia	517
Age of Infants at Onset of Retrolental Fibroplasia	
Relation to Gestational Age and Birth Weight	518
Relation to Time at Removal from Oxygen	518
Weight Gain of Infants and Retrolental Fibroplasia	521
Humidity and Retrolental Fibroplasia	523
Comment	523
Summary	526
Conclusions	528
Recommendations	528
Acknowledgements	528
References	529
Appendix	530

Received for publication July 27, 1956.

From the Kresge Eye Institute (Dr. Kinsey and Mrs. Jacobus) and the Department of Public Health Statistics, School of Public Health, University of Michigan (Dr. Hemphill).

The Cooperative Study of Retrolental Fibroplasia was supported in part by a grant from the National Institute for Neurological Diseases and Blindness of the U. S. Public Health Service, Bethesda, Md.; the National Foundation for Eye Research, Boston, and the National Society for the Prevention of Blindness, New York.

The Coordination Committee was composed of the following members: V. Everett Kinsey, Chairman, Kresge Eye Institute; Richard L. Day, State University of New York College of

(*Footnotes continued on next page*)

Introduction

Administration of oxygen to premature infants had been implicated as an etiologic agent in the development of retrolental fibroplasia (RLF) prior to late 1952.* But because of the apparently capricious fluctuation in incidence of the disease observed in various communities and the insufficiency of controlled investigations concerning the possible role of oxygen in RLF the evidence was deemed inadequate to justify curtailment of oxygen for premature infants, particularly in the absence of knowledge of the effect on mortality of such a change in procedure.

For these reasons a number of pediatricians and ophthalmologists agreed early in 1953 to pool their nursery facilities and interests and investigate cooperatively the question of whether oxygen was in fact an etiologic agent in RLF and at the same time to determine what effect restricting oxygen might have on infant mortality. This group of investigators, from 18 hospitals located in various cities in the United States east of the Mississippi River, began a clinical trial July 1, 1953, which continued until June 30, 1954. A description of this trial and the results obtained are presented in the present report.

An outline of the organizational background of the study and a brief review of the literature on RLF, particularly with respect to etiology, have been published in a preliminary report,[8] which also contains some of the results obtained during the first six months of the study. A comprehensive review of the literature on RLF has been published recently by Zacharias.[9] Accordingly, a discussion of these subjects will not be repeated in the present paper.

Medicine, Brooklyn; Franklin M. Foote, National Society for the Prevention of Blindness, New York; F. M. Hemphill, School of Public Health, University of Michigan, Ann Arbor; Arnall Patz, Johns Hopkins Hospital, Baltimore; Clement A. Smith, Boston Lying-In Hospital; Harold Tager, U. S. Public Health Service, National Institutes of Health, Bethesda, Md.; F. Howell Wright, Chicago Lying-In Hospital.

The following cooperative hospitals and investigators participated in this study: Abington Memorial Hospital, Abington, Pa.: Theodore S. Wilder, M. Luther Kauffman, Robert M. Shelly; Baltimore City Hospital: Harold E. Harrison, David A. Rosen, Laurence Finberg, Carteret Lawrence; Boston Lying-In Hospital: Clement A. Smith, Julian F. Chisholm Jr., Stewart H. Clifford, Merrill J. King, Murray E. Pendleton; Cooper Hospital, Camden, N. J.: Vincent Del Duca, Ronald M. Bernardin, Harold D. Barnshaw; Chicago Lying-In Hospital: F. Howell Wright, Arlington C. Krause, Albert Dorfman; Michael Reese Hospital, Chicago: Benjamin M. Kagan, Theodore M. Shapira, Julius H. Hess, Alwin C. Rambar, Ralph H. Kunstadter, Harold S. Feinhandler, David S. Kane, Samuel M. Schall, Juan E. Nâvarro; Cincinnati General Hospital: John Fuhs, Daniel V. Jones, Carl A. Koch, Edward A. Wagner; Babies' and Children's Hospital (University Hospitals of Cleveland): John H. Kennell, Anita P. Gilger, Samuel Spector, Robert C. Kirk, Elmer J. Ballintine, Charles Q. McClelland; Children's Hospital, Columbus, Ohio: Thomas E. Shaffer, Morris L. Battles, Arthur M. Culler, Edward W. McCall, Miner W. Seymour, John P. Riepenhoff; Charity Hospital, New Orleans: Ralph V. Platou, James H. Allen, E. Sailors, Elaine Allen, Bonnie Adair, Elaine Fichter, George Ellis, Nelson K. Ordway, George M. Haik, M. J. Tiller, Timoteo Jiménez; Babies' Hospital, New York: William A. Silverman, Algernon B. Reese, Richard L. Day, A. M. Dellaporta, Josef Stepanik; Bellevue Hospital, New York: Jonathan T. Lanman, Loren P. Guy; New York Hospital: Mary Allen Engle, David H. Baker, Irving Baras, Alan Freemond, S. Z. Levine; Hospital of the University of Pennsylvania, Philadelphia: Fred H. Harvie, Harold G. Scheie, Harold S. Medoff, Richard A. Ellis; Pennsylvania Hospital, Philadelphia: Henry F. Lee, M. Luther Kauffman; Temple University Hospital, Philadelphia: Norman Kendall, Glen G. Gibson, Arthur A. Bobb Jr.; Elizabeth Steel Magee Hospital, Pittsburgh: E. R. McCluskey, Robert H. Davies, Murray F. McCaslin; Walter Reed Army Medical Center, Washington, D. C.: Ogden C. Bruton, John H. King Jr., William C. Owens.

*References 1-7.

In the preparation of this report it was believed that the interests of pediatricians and other investigators would be served best by including much of the raw data obtained during the study, rather than presenting only derived or average values. While this method necessarily results in numerous and sometimes complex tables and figures, it enables the reader to evaluate the conclusions reached, affords him a basis for further speculation, and allows a more exact comparison with the results of other investigators.

The various relations between incidence of RLF and factors which may influence the incidence are, for the most part, presented graphically, rather than established by formal mathematical procedures. This procedure was followed to establish the relations in a manner which will allow better visualization by the reader.

Purpose

The purposes of the study were (1) to determine whether the incidence of RLF is positively associated with the duration of exposure of the premature infant to an oxygen-enriched environment, (2) to evaluate the effect on mortality of lessening the amount of oxygen given the premature infant after the age of 48 hours, and (3) to obtain information of a general nature concerning the premature infant and his parents that might be of use both in interpreting the data obtained and in formulating any future investigations.

Organization

The Cooperative Study of RLF was conducted under the supervision of a Coordination Committee. Meetings were held from time to time to formulate policy, review progress of the study, and provide general counsel on various aspects of the study.

A center was established at the Kresge Eye Institute, Detroit, to coordinate the efforts of the personnel of the cooperating hospitals. The function of this center was to execute the policies of the Coordination Committee and, in general, to perform those functions which were not carried out by the cooperating hospitals.

Assignment of the infants to the oxygen groups in the clinical trial was made by the Coordination Center. Data obtained by the various hospitals were sent to the Center at weekly intervals, where they were compiled, checked for completeness and accuracy, tabulated, and eventually analyzed.

Data on mortality in all of the hospitals were tabulated at the Center on a week-by-week basis to insure that the recommended procedures with respect to curtailment of oxygen were not accompanied by decreased survival.

Over and above the specific functions of the Coordination Center, the existence of such a center provided an available source of day-to-day information on any problems which might arise within the hospital. It also served to maintain close contact with the responsible investigators, the residents, and the nurses who contributed to the program, and generally to maintain interest and enthusiasm in the study.

Finally, the Coordination Center had the cooperation of the Department of Public Health Statistics, School of Public Health, University of Michigan, where the necessary facilities for statistical analyses were available and much of the data was tabulated.

Design

The plan of this study was to administer oxygen to one group of premature infants for approximately 28 days (a procedure which at the time the study was undertaken was considered routine in premature-infant care) and to restrict the administration of oxygen in a second group of infants to as little as was compatible with the clinical condition of the infant. The first group of infants will be referred to as the "routine-oxygen group," and the second group, the "curtailed-oxygen group." These two groups of infants were to be maintained in all other respects under as nearly similar conditions as possible.

In designing this clinical trial, special consideration was given to two possibilities: (1) that the administration of substantially lesser amounts of oxygen than was customary (as would be the case for the curtailed-oxygen group) might be associated with a significant increase in mortality; (2) that the continued use of the customary amounts of oxygen (as would be the case for the routine-oxygen group) might result in an unnecessarily high over-all incidence of RLF.

With reference to the second possibility, it was considered important to design the clinical trial so that by anticipating a potentially beneficial effect of restricting oxygen any such benefit could be provided currently to the infants in the study population without lessening the possibility of arriving at a decision having statistical validity.

The question of mortality required a design which would permit infants to be assigned to the curtailed-oxygen group in such a way that any difference in mortality rates in the two oxygen groups could be ascertained early in the clinical trial.

Considering the design first from the point of view of conservation of sight, the minimum number of infants who would have to be assigned to the routine-oxygen group to establish any difference in incidence between the routine- and curtailed-oxygen groups, for a given confidence level, would depend upon the number of infants expected to be in the study, the incidence rates anticipated in the two oxygen groups, and the relative number of infants to be assigned to each of these groups. The larger the difference in incidence rates between the infants in the routine- and curtailed-oxygen groups, the more disproportionate would be the contribution of infants in the routine-oxygen group to the total number of infants who might develop RLF. Conversely, the smaller the difference in incidence rates, the smaller would be the relative contribution of the infants of the routine-oxygen group to the total blind population and, furthermore, the less the need for minimizing the number of infants to be assigned to the routine-oxygen group.

To calculate the minimum number of infants to be assigned to the routine-oxygen group for the confidence level selected, i. e. 0.01,† and for varying numbers of infants in a study population, the relative rates of incidence in the two oxygen groups were estimated. Data from previous studies indicated that the incidence rate for the infants in the routine-oxygen group might lie between 10% and 20%. The available information was insufficient for making a realistic estimate of the incidence in the curtailed-oxygen group. Were the calculations to be based on an assumed incidence rate of zero (i. e., the maximum possible beneficial effect of restricting oxygen), the number of infants who would have to be assigned to the routine-oxygen group would be minimal. However, if the incidence rate in the

† The use of the word significant in this report will be limited to confidence levels of 0.01 or less, unless otherwise stated.

curtailed-oxygen group were found to be appreciably higher than zero (i. e., more nearly like that in the routine-oxygen group) there would be a danger that insufficient numbers of infants would have been assigned to the routine-oxygen group to permit conclusions having statistical validity. Accordingly, a low (2%), but nevertheless positive, incidence rate was assumed for the curtailed-oxygen group. This value, considering the method of assignment (to be described later) from a time standpoint, was thought to provide adequately for the safety of the infants yet probably permit a decision within the contemplated year's study. The relative number of infants to be placed in the two oxygen groups for varying-sized total populations was then computed on the basis of these estimates of incidence. (In practice, of course, a few additional infants had to be assigned to the groups to take into account expected losses due to death and inability to follow some infants long enough to make a diagnosis.)

The results of the calculations of the number of infants who would have to be assigned to the routine-oxygen group for varying total numbers of infants are shown in Figure 1. The top and bottom lines correspond to the assumed incidence

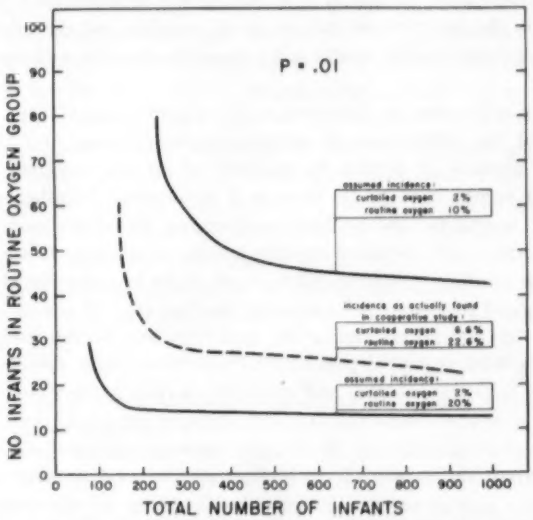


Fig. 1.—Relation of the number of infants who would have to be assigned to the routine-oxygen group to the total number of infants who would be in the study, which would permit conclusions at the 1% confidence level, assuming the incidence rates of RLF to be those indicated in the blocks shown on the chart.

rates of 10% and 2%, and 20% and 2%, in two oxygen groups. In both examples, the number of infants who would have to be assigned to the routine-oxygen group decreases rapidly with the number of infants in the total population. With larger numbers in the total population, the number who would have to be assigned to the routine-oxygen group falls less rapidly but nevertheless decreases slightly up to a population as large as that expected in the Cooperative Study throughout the year (approximately 750 infants). The middle broken line of the Figure, computed in retrospect, is based on the actual incidence figures found in the study and will be discussed later.

Assuming a total available population of about 750 infants and incidence rates closely similar to those used to prepare the graph, in order to obtain significant

results the routine-oxygen group would have to consist of approximately 50 surviving infants who were followed long enough to make a diagnosis. The graph indicates that were all of these 50 infants assigned to the routine-oxygen group within, say, the first three months, when the total number of infants expected to be in the study was approximately 175, estimates of differences in the incidence rates having reasonable statistical validity could be made early, thus permitting a decision as to whether it would be necessary to assign more infants to the routine-oxygen group throughout the remainder of the year.

Accordingly, a decision was made to assign one infant to the routine-oxygen group for each two infants assigned to the curtailed-oxygen group, until such time as 68 infants ‡ had been assigned to the routine-oxygen group; thereafter, all additional infants would be assigned to the curtailed-oxygen group until the incidence rates in the two groups could be evaluated (at approximately six months). It was recognized that this method of assignment would not provide for ideal temporal control. However, in the absence of any evidence of seasonal variation of RLF, the advantages of this design were thought to outweigh greatly the disadvantages. In the event there were an appreciable association between RLF and season, comparative results obtained for the infants in the routine- and curtailed-oxygen groups during the first three months would still permit conclusions with respect to the effect of oxygen.

The design will now be discussed with respect to mortality. Considering the possibility that the restriction of oxygen might increase mortality, in order to minimize the number of deaths the number of infants assigned to the curtailed-oxygen group would have to be kept to a minimum. In other words, from the standpoint of mortality, the problem concerning the distribution of infants between the routine- and curtailed-oxygen groups was opposite to that involved in minimizing the number of infants whose eyes might be deleteriously affected. However, the proposed procedure of assigning the majority, if not all, of those infants to the routine-oxygen group during the first trimester of the study obviated much of the difficulty with regard to problems of mortality, since sufficient infants would be available in both the routine- and curtailed-oxygen groups during the early part of the clinical trial to estimate the mortality rates in the two groups, week by week.

This method of distributing the infants between routine- and curtailed-oxygen groups had three advantages: 1. It minimized the total number of infants to be assigned to the routine-oxygen group, thus tending to minimize total potential blindness. 2. It afforded the opportunity to adjust the ratios of infants in the two groups so as to arrive at a decision having statistical significance within the contemplated one year of the study, should the incidence rates vary considerably from those estimated. 3. It made it possible to determine early whether any difference existed in mortality rates which would suggest altering the procedure of administering oxygen.

Protocol

All premature infants who weighed 1500 gm. or less at birth and who survived 48 hours, born in or brought to the cooperating hospitals, were to be admitted to the study. The Coordination Center was notified by telegram § of the entry of each premature infant, with the date of birth, birth weight, and hospital record

‡ The assumption was made that 18 infants would be lost because of death or lack of follow-up.

§ This procedure was suggested by Dr. Bradford Hill.

number. Through the cooperation of the Western Union Telegraph Company, which supplied the Coordination Center with a teletype machine at no cost, telegrams received during week ends were forwarded to the home of the person in charge of assignments so that the procedure could be carried out on a seven-day week basis. Centralization of assignments at the Coordination Center removed the possibility of any local bias in making the assignment.

Until 68 infants had been assigned to the routine-oxygen group, assignment to the groups was made in sets of three infants, separately for each hospital, where one infant was assigned to the routine-oxygen group for every two infants assigned to the curtailed-oxygen group. The order of assignment was randomized within each set of three infants, and, because of the possibility that the susceptibility of the individual infant to RLF might vary inversely with birth weight, the infants forming each set of three were selected to have approximately the same birth weights. The birth weight categories employed were 1000 gm. and less, 1001 to 1250 gm., and 1251 to 1500 gm. This procedure increased the likelihood that the distribution of susceptibility of the infants in the routine- and curtailed-oxygen groups would be similar. Subsequent to the assignment of 68 infants to the routine-oxygen group all additional infants were assigned to the curtailed-oxygen group.

The infants in the routine-oxygen group were to receive oxygen in concentrations of over 50% for 28 days. The curtailed-oxygen group was to receive added oxygen only if, in the pediatrician's opinion, the clinical condition of the infant demanded it. The concentration of oxygen to be used in this latter group, however, was not to exceed 50%, since it was believed that concentrations in the range of 40% to 50% would be adequate for relief of anoxia. It was thought that the differences in oxygen concentrations employed for the routine- and curtailed-oxygen groups would accentuate differences in incidence.

Orders were to be given to observe the following conditions:

Measure the concentration of oxygen three times a day with a paramagnetic analyzer (Beckman Instruments, Inc.).

Return the infant who received routine oxygen to an air environment at or after 28 days by reducing the flow rate of oxygen by one-third over each of 3 successsive days.

Maintain all controllable conditions, except those related to oxygen administration, as nearly the same as possible in the two groups.

Use the same type of incubator for all infants in any one set of three and use the same schedule for transferring the infants from incubators to bassinets for infants assigned to the two oxygen groups.

Stabilize body temperature in infants of the two oxygen groups at the same level within the range of 90 to 99 F.

Maintain light and humidity as similar as possible in the two groups.

Use mist or wetting agent for all infants of a set of three if required for any one infant. Measure humidity once a day with a Serdex hygrometer.

Use either cow's milk formula or breast milk. In the latter instance, use breast milk for all infants in both oxygen groups for the same duration, and change formula at the same time to a similar one for infants in each set.

Use the following formula:

Evaporated milk	100 ml.
Water	200 ml.
Cane sugar	15 gm.

Administer vitamins starting not later than one to two weeks after birth. Use nonoily preparation containing vitamins A, C, and D, but no vitamins E or B, in the following dosage (after an introductory period of a few days):

To each 300 calories of the formula add 0.8 ml. of a preparation of vitamins A, C, and D such as Tri-Vi-Sol (Mead Johnson & Company) or A-D-C drops (Parke, Davis & Company). Use no carbohydrate other than cane sugar because, among other reasons, of varying salt content of other preparations.

(This prescription contains 6.8% carbohydrate in terms of reconstructed evaporated milk and has a caloric density of 20 Cal. per ounce. To every 100 Cal. of formula add 3530 units of vitamin A, 32 mg. of vitamin C, and 680 units of vitamin D).

Administer vitamin K subcutaneously to all infants upon admission to the nursery, if not previously administered; dosage, 2.5 mg.

Give blood transfusions only when clearly indicated clinically. Use the same procedures with regard to therapeutic and prophylactic agents for infants in both the routine- and curtailed-oxygen groups in any one hospital. Prescribe the same antibacterial treatment for infants brought to the hospital as for infants born in a participating hospital for each set of three infants (penicillin and sodium sulfadiazine recommended).

Examine the eyes of the infants ophthalmoscopically at 3, 4, 5, 6, 7, 9, 11, 15, and 25 weeks of age. Forward the results of these examinations to the Coordination Center together with the weekly record sheets while the infant is in the hospital nursery, or separately after discharge from the nursery. Classify results of the ophthalmoscopic examination according to numerical evaluation of the active stage and alphabetical evaluation of the cicatricial grade of the disease, as described by Reese, King, and Owens[10] and reproduced in Table 1A (Table 1 of the Appendix).

Record and forward to the Coordination Center the specific information concerning both the premature infant and the parents upon admission of an infant to the study. (The record form is illustrated in Figure 1A. [Figure 1 of the Appendix]. The items which were to be reported on a weekly basis are shown for infants in each of the oxygen groups in Figure 2A. The latter information was to be mailed to the Coordination Center at weekly intervals.)‖

Population

From July 1, 1953, to June 30, 1954, a total of 786 premature infants,¶ who weighed 1500 gm. or less at birth, were born in or brought to the 18 cooperating hospital nurseries, survived 48 hours, and were admitted to the study at this age. Of these infants, 166 died prior to 40 days # of age. Of the remaining 620 infants, 586, or 95%, were followed for a minimum of two and a half months. (Actually, the

TABLE 1.—*Disposition of Infants Admitted to the Study (Age Forty-Eight Hours)*

	Infants, No.
Total admitted	786
Died prior to 40 days of age	166
Survived 40 days	620
Survived 40 days; eyes examined for a minimum of 2½ mo.	586

‖ Record keeping in each participating hospital was performed by the responsible investigators, residents, and nurses and, in some instances, part-time employees. When the study was initiated, the idea of assigning a full-time record-keeper to each hospital was considered; it was discarded as being unnecessarily costly in view of the small number of babies in many of the hospitals. Probably this method of keeping records would have been a more efficient way of collecting data. Also, it might have been helpful to employ a person to visit the various hospitals at frequent intervals, to insure accuracy and completeness of record-keeping. This might have eliminated much correspondence and telephoning by the Coordination Center to complete and, in some instances, to correct the records. In addition, this same worker could have assisted in instructing new nurses and residents in those hospitals where rotation among services occurred as frequently as every three months.

¶ There were 634 additional premature infants in this birth-weight category born in or brought to these hospital nurseries during this period. All of these were reported to have died before 48 hours.

It is presumed that after 40 days of age any deaths that occurred were unrelated to curtailment of oxygen.

majority of infants were followed for six months; less than 5% were followed for less than four months. In all but three infants in the latter group, the eyes were normal when last examined, and therefore the majority of the eyes in this group may be presumed to have remained normal. In one infant the eyes already had shown signs of cicatricial RLF, and in the other two the eyes showed signs of active Stage 1 of RLF at the time of the last examination.) The group of 586 infants will hereinafter be referred to as the study population (Table 1). Of the 34 infants (5%) who were not followed for two and one-half months or longer, 7 died and 27 others could not be followed for one reason or another.

The race, weight group, place of birth, and oxygen grouping of the 586 infants in the study population are shown in Table 2A, classified by hospital and separated by single and multiple birth.* The same information for the 166 infants who died prior to 40 days of age is shown in Table 3A, and similar data, not separated by hospital, are shown in Table 4A for those 34 infants who were not in the study population either because of death (7 infants) or lack of follow-up (27 infants). Examination of the available records of these 34 infants revealed no trends which would suggest disproportionate differences in ocular findings when compared with those infants of the same age in the whole study population.

Mortality† in the Routine- and Curtailed-Oxygen Groups

Of the 68 infants in the routine-oxygen group, 15 died prior to 40 days of age, a mortality of 22%; of the 144 infants in the curtailed-oxygen group during the same time period (the first three months of the study only), 36 died, a mortality of 25% (Table 2). This difference in mortality rate is not statistically significant.

TABLE 2.—*Mortality* of Infants Admitted to the Study (Age Forty-Eight Hours)*

Oxygen Group	Trimester of Study	Mortality		
		Single Births, %	Multiple Births, %	Total, %
Routine	1	21.7 (13/60)	25.0 (2/8)	22.0 (15/68)
Curtailed	1	25.9 (30/116)	21.4 (6/28)	25.0 (36/144)
Curtailed	2	16.9 (26/154)	3.8 (1/26)	15.0 (27/180)
	3	20.4 (27/132)	12.8 (5/39)	18.7 (32/171)
	4	25.4 (46/181)	23.8 (10/42)	25.1 (56/223)
Curtailed	1-4	22.1 (129/583)	16.3 (22/135)	21.0 (151/718)
Routine and curtailed		22.1 (142/643)	16.8 (24/143)	21.1 (166/786)

*Infants who died prior to 40 days of age.

During the entire year of the clinical trial 718 infants were assigned to the curtailed-oxygen group; 151 of these infants died (prior to 40 days), a mortality of 21% (Table 2). This rate is likewise not significantly different from the 22% observed for the routine-oxygen group.

* Analysis of the data indicated a striking difference in the incidence of RLF between infants of single and those of multiple birth. Since the proportion of single and multiple births in any particular group of infants studied will therefore greatly influence the results, most of the data will be presented separately by these categories.

† All figures refer to mortality after admission to the study, at 2 days of age.

The mortality rates among infants of single and multiple birth in both the routine- and curtailed-oxygen groups also do not differ significantly.

The mortality rates for routine- and curtailed-oxygen groups within each participating hospital nursery are shown in Table 5A. The variations in mortality between the two oxygen groups in the various hospitals are no greater than would be expected, considering the number of infants involved in any one hospital and the probable effect of varying ratios of in- and outborn infants in the different hospitals.

That the infants assigned to the routine- and curtailed-oxygen groups were in fact exposed to widely different periods in oxygen is shown in Figure 2.‡ The average stay in oxygen was 30 days for infants in the routine-oxygen group, and

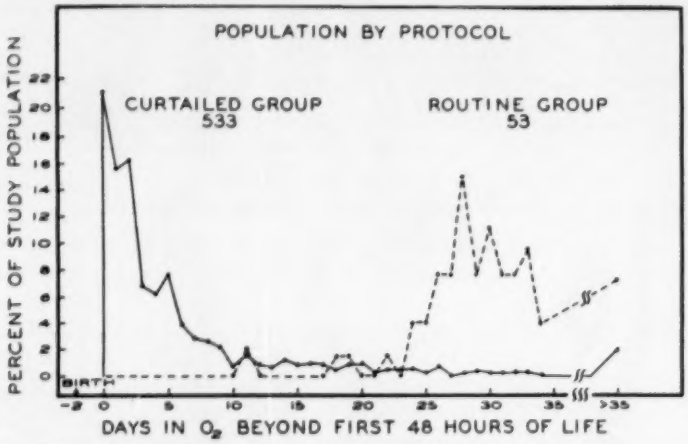


Fig. 2.—Distribution of infants according to number of days in oxygen for routine- and curtailed-oxygen groups.

the average stay for infants in the curtailed-oxygen group was 5 days.

The data on mortality rates in the two oxygen groups suggest that reducing the length of stay in oxygen to that deemed necessary to meet acute clinical needs of the infant is without effect on mortality.

Incidence of RLF in Routine- and Curtailed-Oxygen Groups

Active Stages of RLF

The percentage of infants in the routine- and curtailed-oxygen groups who developed active stages of RLF is shown in Table 3 for infants of single and multiple birth according to the maximal stage developed in the poorer eye. The incidence in infants in the curtailed-oxygen group is given separately for the first three months of the study as well as for the whole year in order to permit a comparison with the incidence in infants who were exposed to routine oxygen during the same time period.

‡ Some infants in the routine-oxygen group, who by protocol should have received 28 days of exposure to oxygen, actually were in oxygen for 2 days less than this period (Fig. 2). This deviation from the protocol occurred when the Coordination Center was not notified of the birth of an infant soon enough to make the assignment within 48 hours after birth. There were three infants who received 11, 18, and 19 days of oxygen, respectively; two instances were due to errors and in one the infant was removed from oxygen because of illness. These exceptions to the protocol could only have decreased the difference in incidence observed between infants in the routine- and curtailed-oxygen groups.

TABLE 3.—*Incidence of Active Stages of Retrolental Fibroplasia* According to Oxygen Group*

RLF Stage	Routine-Oxygen Group (3 Mo.)			
	Single Births (47 Infants)		Multiple Births (6 Infants)	
	% Incidence	Infants, No.	% Incidence	Infants, No.
1	25.5	12	33.3	2
2	23.4	11	16.7	1
3	12.8	6	16.7	1
4	6.4	3	16.7	1
5	2.1	1	0	0
Total 1-5	70.2	33	83.3	5
	Curtailed-Oxygen Group (12 Mo.)			
	Single Births (425 Infants)		Multiple Births (108 Infants)	
1	18.1	77	25.9	28
2	8.2	35	3.7	4
3	3.3	14	6.5	7
4	1.2	5	3.7	4
5	0.5	2	1.9	2
Total 1-5	31.3	133	41.7	45
	Curtailed-Oxygen Group (1st 3 Mo.)			
	Single Births (82 Infants)		Multiple Births (22 Infants)	
1	15.8	13	22.7	5
2	7.3	6	4.5	1
3	6.1	5	9.1	2
4	0	0	4.5	1
5	0	0	4.5	1
Total 1-5	29.3	24	45.4	10

*Maximal stage developed in poorer eye.

Actually, the incidence in infants of single birth and also in those of multiple birth in the curtailed-oxygen group (upper part of Table 6A) did not vary significantly from trimester to trimester throughout the year and hence was not seasonally dependent. Thus, it appears valid to compare the incidence in the routine-oxygen group with that found in the curtailed-oxygen group for the entire 12-month period.

Table 3 shows that of the 425 infants of single birth who were in the curtailed-oxygen group during the year about one-third showed some active stages of the disease, compared with over two-thirds of the 47 infants of single birth in the routine-oxygen group. This difference in incidence in oxygen groups is significant.

Of the 108 infants of multiple birth in the curtailed-oxygen group, about two-fifths showed some active stage of the disease compared with five-sixths of the 6 infants in the routine-oxygen group ($P < 0.05$).

Not only was the incidence rate of the active stages slightly higher among the

infants of multiple birth, but relatively more infants of multiple birth showed signs of the advanced stages of the disease than those of single birth. This fact is associated with a difference in spontaneous regression rates between the two groups, a subject which will be discussed later in this report.

Cicatricial Grades of RLF

The percentage of infants who developed cicatricial grades of RLF (one or both eyes) in the routine- and curtailed-oxygen groups is shown in Table 4. The incidence of RLF in infants in the curtailed-oxygen group is shown separately for the first three months of the study as well as for the full year. There was no significant variation from trimester to trimester (Table 6A, lower part).

TABLE 4.—*Incidence of Cicatricial Grades of Retrolental Fibroplasia* According to Oxygen Group*

RLF Grade	Routine-Oxygen Group (3 Mo.)			
	Single Births (47 Infants)		Multiple Births (6 Infants)	
	% Incidence	Infants, No.	% Incidence	Infants, No.
A	2.1	1	16.7	1
B	4.2	2	0	0
C	2.1	1	16.7	1
D	4.2	2	0	0
E	4.2	2	33.3	2
Total A-E	17.0	8	66.7	4

	Curtailed-Oxygen Group (12 Mo.)			
	Single Births (425 Infants)		Multiple Births (108 Infants)	
A	2.1	9	2.8	3
B	1.2	5	0.9	1
C	0	0	4.6	5
D	0.7	3	2.8	3
E	0.7	3	2.8	3
Total A-E	4.7	20	13.9	15

	Curtailed-Oxygen Group (1st 3 Mo.)			
	Single Births (82 Infants)		Multiple Births (22 Infants)	
A	2.4	2	0	0
B	1.2	1	0	0
C	0	0	4.5	1
D	1.2	1	4.5	1
E	0	0	9.1	2
Total A-E	4.9	4	18.2	4

*Maximal grade developed in poorer eye.

Table 4 shows that approximately one-sixth of all the infants of single birth in the routine-oxygen group developed some grade of cicatricial RLF, compared with about one-twentieth of those in the curtailed-oxygen group who developed the disease.

Two-thirds of the infants of multiple birth in the routine-oxygen group showed some grade of cicatricial RLF, compared with one-seventh in the curtailed-oxygen group. The difference in incidence between the two oxygen groups is significant.

Over and above the differences in incidence in these two groups, the Table shows the striking difference in incidence of RLF in infants of single and of multiple birth. This observation has both theoretical and practical importance and will be discussed later.

While the number of infants involved in some of the hospitals was so small that no significant association between oxygen and RLF could be demonstrated in any particular hospital, the results obtained suggest that the association was not geographically dependent (Table 7A).

Comment

The results of this controlled clinical trial would seem to constitute a clear-cut prima-facie demonstration of a positive association between the incidence of RLF and the exposure of the premature infant to oxygen and also a demonstration that the mortality rate was unaffected when oxygen was used not routinely but only as a specific treatment for certain clinical needs of the infant. The significance of the results, however, would be materially affected were factors which have been thought to influence susceptibility of the infant to RLF, or mortality, such as gestational age, birth weight, and health status, not distributed proportionately between the two oxygen groups, despite the use of procedures expected to bring this about.

Table 5 shows that the gestational ages and birth weights of the infants assigned

TABLE 5.—*Average Gestational Age and Average Birth Weight of Infants in Routine- and Curtailed-Oxygen Groups*

Oxygen Group	Av. Gestational Age				Av. Birth Weight			
	Single Births		Multiple Births		Single Births		Multiple Births	
	Wk.	Infants, No.	Wk.	Infants, No.	Gm.	Infants, No.	Gm.	Infants, No.
Routine	32	36	32	4	1,234	47	1,287	6
Curtailed (12 mo.)	31	344	33	86	1,242	425	1,278	108
Curtailed (1st 3 mo.)	31	68	31	13	1,260	82	1,285	22

to the routine- and curtailed-oxygen groups were essentially the same, when a comparison is made on the basis of both those infants admitted during the first trimester and those during the entire year of the study. The health status of the infants in the two oxygen groups can be assessed only in a relatively quantitative manner. Inspection of the records revealed no conspicuous differences in the two groups, and in view of the similarity in the average weight gain (Table 21) it seems probable that the proportion of "sick" infants in the two oxygen groups was also approximately the same.

Thus, because these factors which might have influenced the incidence of the disease or the mortality were actually distributed proportionately between the two oxygen groups, the likelihood of a cause and effect relation existing between dura-

tion in oxygen and RLF is enhanced. Moreover, the conclusion appears justified that mortality was unaffected when oxygen was restricted to amounts deemed necessary to prevent anoxia.

In designing this clinical trial every effort was made to insure that if indeed oxygen were positively associated with RLF this fact would be conclusively established. The procedure recommended to accomplish this was to expose the two groups of infants to oxygen for widely different periods of time and to distinctly different concentrations. Since there was a highly positive association between exposure to oxygen and incidence of RLF, it appeared profitable to attempt to establish a more quantitative relation between both incidence and severity and conditions of exposure to oxygen. Furthermore, while some factors, such as multiplicity of birth, health status of the infant, gestational age, and, to a lesser extent, birth weight (because of the use of the three birth weight categories in the assignment procedure), were not controlled in the clinical trial, the data collected can be analyzed to study the possible influence of these factors on the incidence and severity of RLF for any given exposure to, or concentration of, oxygen.

An analysis of the data of the Cooperative Study will now be presented to show quantitatively the relation between the per cent incidence, as well as the severity, of RLF and the duration of exposure of the premature infant to oxygen. The manner in which this relation depends upon such factors as multiplicity of birth, health status of the infant, concentration of oxygen employed, gestational age, birth weight, sex, etc., will also be shown. It is believed that the results of these analyses will enable the pediatrician to make a reasonable estimate of the probability that any particular infant will develop RLF as a result of a given exposure to oxygen and thus serve as a useful guide in weighing the risk involved in prescribing oxygen for those infants whose poor clinical condition may justify its use.

Multiplicity of Birth and Incidence of RLF

The incidence of active and cicatricial RLF in infants of single and multiple birth,§ as well as the average duration in oxygen, concentration of oxygen, gestational age, and birth weights of these groups of infants, are shown in Table 6. The per cent incidence of the active form of RLF in infants of multiple birth is one and a quarter times greater than that in infants of single birth, whereas that of the cicatricial disease was almost three times greater in infants of multiple birth

TABLE 6.—*Incidence of Active Stages and Cicatricial Grades of RLF, Average Duration in Oxygen, Average Concentration of Oxygen, Average Gestational Age, and Average Birth Weight of Infants of Single and Multiple Birth*

Birth	RLF		Av. Duration in O_2,* Days	Av. Conc. of O_2,* %	Av. Gest. Age, Wk.	Av. Birth Weight, Gm.
	Active Stages, %	Cic. Grades, %				
Single	35.2 (166/472)	5.9 (28/472)	8†	39	31	1,240
Multiple	43.8 (50/114)	16.7 (19/114)	6	40	33	1,280

*Figures include those infants who did not receive oxygen.
†For number of infants from which these average values are computed, see Tables 8, 10, 11, 12, and 13.

§ One hundred eleven of the infants of multiple birth were of twin birth, and three infants were of triplet birth.

than in those of single birth. The difference between the rates of active stages of RLF for the two groups is not significant (at the 0.01 level), whereas that between the cicatricial rates is highly significant.

Differences in the relative incidence rates of active and cicatricial RLF for infants of single and multiple birth suggest that infants of multiple birth are less resistant to the further development of the disease, in other words that their rate of spontaneous regression is considerably less than that of infants of single birth. Quantitative interpretation of the results with respect to possible differences in susceptibility or resistance to continued development of the disease is complicated, however, since not only was the average time of exposure to oxygen different in the infants of single and multiple birth but the infants differed also with respect to factors other than multiplicity of birth. The Table shows that the average number of days in oxygen was greater for infants of single than of multiple birth. Moreover, the average gestational age and birth weight of infants of single birth were less than those of infants of multiple birth. Were all three of these factors more nearly alike for infants of single and multiple birth, presumably the difference in incidence would be greater than observed. Thus, the true difference in both susceptibility to the active disease and resistance to the development of the cicatricial form may be greater than the incidence figures in Table 6 would indicate.

Since multiplicity of birth seems to be such an important factor in determining the incidence of RLF for any given exposure to oxygen, any potential effect of other factors investigated, such as birth weight, gestational age, etc., would be readily masked were infants of single and multiple birth not equally distributed among groups formed to study these other factors. Most of the data, therefore, will be presented separately for infants of single and multiple birth.

Health Status of the Infant and Incidence of RLF

The clinical condition of the infant is one factor which has been thought to affect the incidence of RLF, the consensus being that infants who do not progress well in the nursery might be more susceptible to the disease.

Actually, it is possible that poor clinical progress, thought (in the past, at least) to require longer exposure to oxygen, may be negatively rather than positively associated with RLF. The degree of saturation of oxygen in the blood delivered to the retina of an infant who is receiving added oxygen, but who shows signs of pulmonary insufficiency, may not exceed, and may actually be less, than that of a "well" infant in an air environment. Considered from this point of view, the positive association frequently observed between RLF and those infants in a hospital nursery who had the longer stays in oxygen, who, therefore, were presumed to represent the sicker segment of the population, might have been even more conspicuous than was observed.

Studies designed to determine what effect, if any, the clinical condition of the infant has on the incidence of RLF have been hampered by difficulty in dissociating poor progress in the nursery from the length of stay in oxygen, since these factors ordinarily go together. It is clear that until the factors of use of oxygen and poor clinical progress can be separated it is not possible to determine what effect if any the latter has on RLF. In this study the circumstances coincident with the administration of oxygen to the infants in the routine-oxygen group and one segment of those infants in the curtailed-oxygen group provide a unique situation, where the length of stay in oxygen was essentially the same in the two groups of infants while their health status was probably very different.

With one exception, all of the infants in the routine-oxygen group were given oxygen for 15 or more days. This group presumably contained no greater proportion of sick infants than was to be found in the study population as a whole, and so, for the purpose of this analysis, this group may be considered the "well"-infant group. By coincidence, the same number of infants in the curtailed-oxygen group also received oxygen for 15 days or more. However, it may be assumed that most, if not all, of these infants were ill, since it was only because of poor clinical condition that they were to receive additional oxygen at all. These infants may therefore be considered as the "sick"-infant group. That the health status of the infants in the two groups was actually different is indicated by the observation that the average weight gain during the first 40 days in the well-infant group was approximately double that in the sick-infant group (Table 7).‖

TABLE 7.—*Incidence of Cicatricial RLF, Average Duration in Oxygen, Average Concentration of Oxygen, Average Gestational Age, Average Birth Weight, and Average Weight Gain of "Sick" and "Well" Infants of Single and Multiple Birth Who Received Fifteen or More Days of Oxygen*

Health Status	Incidence RLF, %	Av. Duration in O_2, Days	Av. Conc. of O_2, %	Av. Gest. Age, Wk.	Av. Birth Weight, Gm.	Av. Weight Gain,* Gm.
Single births						
"Well" infants	17 (8/47)	30	54	31	1,240	490
"Sick" infants	8.5 (4/47)	25	41	30	1,075	200
Multiple births						
"Well" infants	80 (4/5)	31	54	31	1,240	500
"Sick" infants	50 (3/6)	28	39	27	952	250

*First 40 days.

The incidence rates of cicatricial RLF in the two groups are shown in Table 7, along with the average durations in oxygen, concentrations of oxygen, gestational ages, birth weights, and weight gain during the first 40 days of life. The Table shows that for essentially the same duration in oxygen the incidence in the well-infant group is approximately twice that in the sick-infant group, for infants of both single and multiple birth, despite the fact that the average birth weight was considerably lower in the sick-infant group. The average concentration of oxygen, on the other hand, was higher in the well-infant group. This observation might suggest that the higher incidence of RLF in the well-infant group could be accounted for by differences in the concentration of oxygen rather than by differences in health status. However, it will be shown subsequently that concentration differences of this magnitude seem to have little, if any, effect on the incidence of the disease. Poor clinical progress thus seems to be negatively rather than positively correlated with RLF, a conclusion consistent with the reasoning based on the hypothesis that the oxygen saturation level of the blood is the ultimate determining factor associated with RLF and that the level may be appreciably lower in the sick-infant group for a given exposure to oxygen.

Duration of Oxygen and Incidence of RLF

To determine the relation between the duration of exposure of the infant to oxygen and the incidence of RLF, the population of infants was divided so as to

‖ Differences in the average birth weight in the two groups would be expected to account for only about 15% of the observed differences in average weight gain.

form groups of infants who had similar periods of exposure to oxygen.¶ The incidence of active stages and of cicatricial grades of RLF was computed for each of these groups; the relation between the incidence of RLF and length of exposure to oxygen for infants of both single and multiple birth is shown in Figures 3 and 4 for the two phases of the disease, respectively.

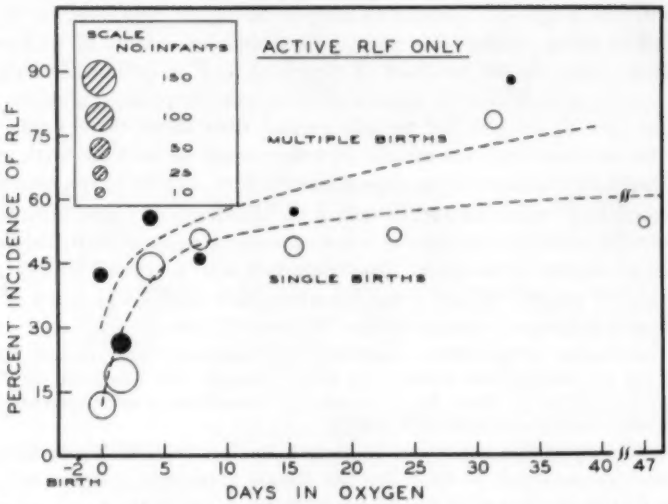


Fig. 3.—The relation between incidence of active RLF and number of days the infants were in oxygen after admission to the study. Open circles refer to infants of single birth; filled circles refer to infants of multiple birth.

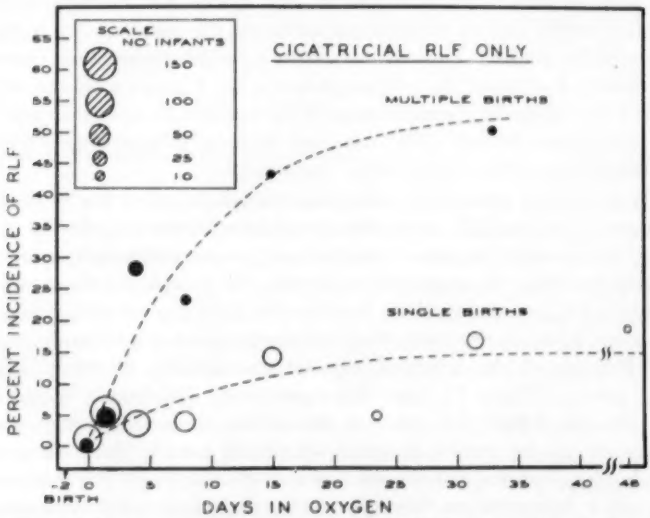


Fig. 4.—The relation between incidence of cicatricial RLF and number of days the infants were in oxygen after admission to the study. Open circles refer to infants of single birth; filled circles refer to infants of multiple birth.

¶ All of these data refer to period of exposure after entry into the study (age 48 hours), which with few exceptions started immediately after admission.

Figure 3 shows that the incidence of the active stage of the disease increases rapidly with the length of exposure to oxygen for infants of both single and multiple birth, the increase being greatest during the first week of exposure to oxygen. The graph shows also that the incidence is slightly higher for infants of multiple than of single birth.

Figure 4 shows that the incidence of the cicatricial form of RLF also increases rapidly with the length of exposure to oxygen during the first week or 10 days of age, the effect being considerably more pronounced for infants of multiple birth. The difference between the incidence of cicatricial RLF in infants of single and of multiple birth is conspicuous for exposures to oxygen for periods in excess of three days. Exposure to oxygen for periods greater than about three weeks did not increase the incidence rate for infants of either single or multiple birth, the rates for this length of exposure being approximately 15% and 50%, respectively.

Cicatricial RLF was observed in only 1 of 93 infants of single birth and none of 19 infants of multiple birth who were not exposed to an oxygen-enriched environment after admission to the study. The one infant who developed RLF (Grade A, both eyes; birth weight, 907 gm.) was known to have received 11 hours of oxygen (concentration unknown) during the first 48 hours of life.

Some information is available concerning the experience with respect to oxygen for 52 of the 112 infants who received no added oxygen after admission to the study. These data are shown in Table 8A. The average concentration administered was 41%, varying in individual infants from 28% to 60%.

The data suggest that exposures to oxygen for less than 48 hours duration are accompanied by some risk of RLF, but the disease appears to progress to a cicatricial grade less frequently than for longer periods of exposure.

When the study population was divided into groups of infants who had approximately equal lengths of exposure to oxygen, as was done in preparing Figures 3 and 4, no attempt was made to do this in such a way that the magnitude and/or distribution of other factors which might influence the susceptibility to the disease would likewise be similar. Thus to the extent to which these other factors might not be randomly distributed the relation shown in the Figures would be skewed and hence would not really be representative of the relation in a normal population of surviving premature infants. The data can now be examined to determine the extent to which these other factors were randomized.

One factor already considered which was thought to affect the susceptibility of the premature infant to RLF is the clinical condition of the infant. The proportion of sick and well infants in each of the several groups used in preparing Figure 4 was probably unequal. As suggested previously, the groups of infants who had the longer stays in oxygen, except those infants who were to receive oxygen according to protocol, must have contained a disproportionate number who made poor clinical progress. Because of the apparent reduced susceptibility of those infants who progressed poorly (Table 7), this disproportionate distribution would have the effect of reducing slightly the apparent dependence of incidence upon the stay in oxygen, particularly for periods in oxygen exceeding a week. Thus, for a population of infants having a normal distribution as far as clinical progress is concerned RLF is slightly more dependent on time spent in an oxygen-enriched environment than is shown in Figure 4.

Among other factors which earlier studies have suggested may influence the incidence of the disease are concentration of oxygen administered, gestational age, and birth weight. The average value for each of these factors was computed for

groups of infants divided according to approximately equal periods in oxygen and is shown in Table 8. (These groups are similar, but not identical, with those used in

TABLE 8.—*Duration in Oxygen**

Incidence of Cicatricial Retrolental Fibroplasia, Average Concentration of Oxygen, Average Gestational Age, and Average Birth Weight of Infants Grouped According to Stay in Oxygen

Duration in Oxygen	Incidence RLF		Av. Conc. O_2		Av. Gest. Age		Av. Birth Weight	
Days	%		%	Infants, No.	Wk.	Infants, No.	Gm.	Infants, No.
Single births								
0	1.1	(1/93)	20	93	32	77	1,266	93
1-2	5.4	(7/130)	41	119	31	108	1,302	130
3-5	3.5	(3/86)	44	85	31	66	1,272	86
6-10	3.8	(2/52)	44	50	31	38	1,231	52
11-20	14.0	(6/43)	43	43	31	36	1,139	43
21 and more	13.2	(9/68)	50	68	31	51	1,181	68
Average	5.9	(28/472)	39†	458	31	380	1,250	472
Unknown duration	..	(0/0)	..	0	..	0	..	0
Unknown other factors	..	..	..	14	..	92	..	0
Multiple births								
0	0	(0/19)	20	19	35	15	1,325	19
1-2	4.8	(2/42)	40	36	33	35	1,276	42
3-5	28.0	(7/25)	48	24	33	21	1,281	25
6-10	23.1	(3/13)	46	12	33	10	1,354	13
11-20	50.0	(3/6)	48	6	34	3	1,233	6
21 and more	44.4	(4/9)	48	9	29	6	1,114	9
Average	16.7	(19/114)	40†	106	33	90	1,280	114
Unknown duration	..	(0/0)	..	0	..	0	..	0
Unknown other factors	..	..	..	8	..	24	..	0

*After admission to study, age 48 hours.
†Figures include those infants who did not receive any oxygen.

Figure 4.) Values for those infants who did not receive any additional oxygen are also shown, as well as values for the group whose stay in oxygen was unknown.

The average concentrations of supplemental oxygen for the groups of infants of single birth for periods of 1 to 20 days were approximately equal (Table 8, fourth column). For the group of infants who received 21 days or more of oxygen the average concentration was approximately 7% higher, chiefly as a result of the disproportionate number of infants in this group who were assigned to the routine-oxygen group.

The average concentrations of oxygen for the groups of infants of multiple birth who received oxygen for three days and longer were also approximately the same. Moreover, the distribution of the individual average daily oxygen concentrations of the infants comprising the groups in Table 8 were similar.

The average gestational ages for infants of both single and multiple birth did not differ appreciably except for the group of infants of multiple birth who received oxygen for 21 days and more. The individual gestational ages from which the averages were computed were normally distributed in each group. These data

suggest that the observed relation between incidence and increased length of stay in oxygen (Figs. 3 and 4) was not appreciably affected by differences in average concentration of oxygen or gestational age within the groups of individuals used to demonstrate the relation.

The average birth weights for the several groups of infants, however, varied considerably. In general, the infants with the lower birth weights were exposed to oxygen for the longer periods; consequently, if susceptibility to the disease were inversely related to birth weight, as is commonly believed, the relation between duration and incidence would be disproportionately high for the longer time periods.

The following section will be concerned with an attempt to determine the relation between incidence of RLF and duration in oxygen, uninfluenced by differences in birth weight within the various groups of infants used to demonstrate the relation.

The general procedure employed will be described in some detail because the same method of treating the data will be used several times in this report.

The procedure consists in forming groups of infants (single and multiple births, separately) in such a way that the average value for the factor whose effect it is desired to single out (e. g., duration in oxygen) differs widely between groups, while that of another factor (e. g., birth weight) is held substantially equal (at one or more levels of magnitude). This arrangement of the data makes it possible to determine the relation between incidence, for instance, and a first factor, at one or more levels of magnitude of the second factor, without so reducing the numbers of infants in any one group as to make the data statistically weak. The use of only a few groups, however, does result in lessened definition of the relation to be established.

Ideally, it is desirable that the magnitude of the factor whose average value is to be maintained essentially equal, i. e., the second factor, be identical for each individual in the several groups into which the population is divided and that the magnitude of the first factor differ widely from one group to another. Actually, in this study the individual values for the second factor were not equal in most instances, and so groups were formed from individuals who differed with respect to the second factor but whose average value for each group was approximately the same.

In practice, groups of infants were formed by dividing the populations into two groups of about the same size with respect to the average value which was to be kept similar. These groups were then divided into two other groups, again of about equal size, but in this instance having average values widely different with respect to the first factor. In some instances two other groups were formed consisting of those infants who received no added oxygen (making a total of six groups).#

It is important to observe that dividing the study population into groups as indicated above will permit interpretation of the effect of the factor being singled out only if no other factor which may affect the relationship to be demonstrated is distributed differently (either from the standpoint of magnitude or frequency of occurrence) within any of the four or six groups under consideration.

The infants were divided into six groups according to stay in oxygen and birth weight in the manner shown in Table 9, which shows also the incidence of RLF, average gestational age, and average concentration of oxygen for each of the groups. (The distribution of birth weight of the infants within each of these groups is shown in Figures 3A [single births] and 4A [multiple births].) This method of distributing the population gives rise to three groups of individuals comprising the

This latter method of grouping in a sense tends to beg the question in so far as the reason for its use is based on the idea that were there no additional oxygen administered there would be no appreciable risk. However, the addition of two more groups does not distort the data and it appears valid to examine them in this manner.

TABLE 9.—*Incidence of Cicatricial Retrolental Fibroplasia, Average Gestational Age, and Average Concentration of Oxygen of Infants Grouped According to Birth Weight and Duration in Oxygen*

Birth Weight Grouping	Oxygen Grouping	Incidence RLF, %	Av. Gestational Age, Wk.	Av. Concentration O_2, %
Single births				
1,300 gm. and more	No days in O_2	0	33	--
1,299 gm. and less	No days in O_2	2.2	31	--
1,300 gm. and more	1-3 days	2.2	32	40
1,300 gm. and more	4 days and more	5.8	31	46
1,299 gm. and less	1-3 days	8.8	31	41
1,299 gm. and less	4 days and more	10.5	30	46
Multiple births				
1,300 gm. and more	No days in O_2	0	35	--
1,299 gm. and less	No days in O_2	0	35	--
1,300 gm. and more	1 and 2 days	0	34	37
1,300 gm. and more	3 days and more	30	33	46
1,299 gm. and less	1 and 2 days	9.1	32	44
1,299 gm. and less	3 days and more	35	32	47

heavier segment of the population, which had widely different stays in oxygen, and three groups comprising the lighter segment of the population, which likewise had different stays in oxygen.

The relation between the incidence of RLF and duration in oxygen in groups of infants having closely similar birth weights is shown separately for the heavier and lighter segments of the populations for infants of single and multiple birth in Figure 5.

The incidence of RLF in infants of single birth increased rapidly during the first day and a half of exposure to oxygen and thereafter increased but slightly for both the lighter and heavier segments of the population, in part, perhaps, because

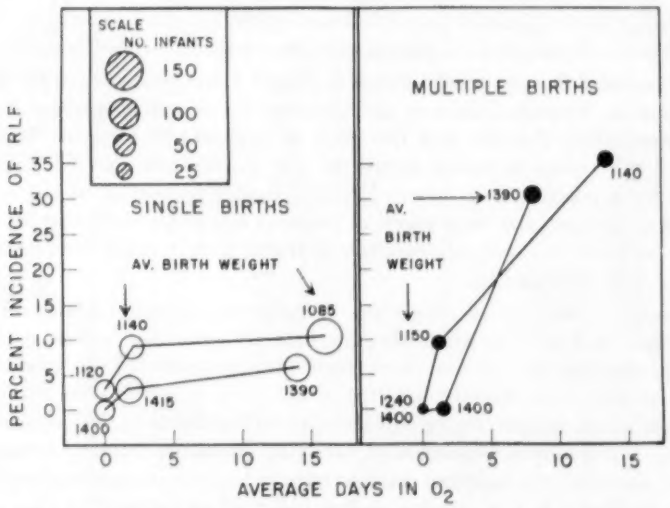


Fig. 5.—The relation between incidence of cicatricial RLF and average number of days groups of infants having similar birth weights were in oxygen after admission to the study.

the groups of infants having the longer stay in oxygen contained a disproportionate number of sick infants. The incidence of RLF in infants of multiple birth increased throughout the stay in oxygen.

The incidence of RLF for infants of single birth was considerably higher in the lighter than in the heavier segment of the population for all durations in oxygen. Differences in birth weight did not appear to affect greatly the incidence in infants of multiple birth. Subsequently in this report the effect of birth weight on susceptibility to the disease will be discussed in detail.

It will be recalled that the gestational ages and average concentrations of oxygen for the various groups of individuals shown in Figure 4 were essentially the same. Table 9 shows that the gestational age was also essentially the same in both the lighter and heavier groups of infants employed in Figures 3A, 4A, and 5. However, the average concentration of oxygen to which the population of infants was exposed was not the same in those groups of infants who received added oxygen, i. e., four of the six groups. In general, the average concentration was higher for those infants whose stay in oxygen was the longer; hence any increased incidence observed with the longer stays in oxygen might be due to a concentration effect. However, in the case of both the lighter and heavier segments of the population of infants of single birth, the incidence was only slightly higher in the infants exposed to approximately 15 days of oxygen than in those exposed to 1 to 3 days of oxygen, and so differences in concentration could have exerted little, if any, effect on the incidence rate.

Table 9 shows that there was no appreciable difference in the average concentration of oxygen administered to the two groups composing the lighter segment of the population of infants of multiple birth (44% and 47%). In the heavier segment of the population of infants of multiple birth the average concentration differs by 9% (37% and 46%) and could in part account for the increased incidence with longer exposures to oxygen. However, since the relation between incidence and average days in oxygen in this heavier segment of the population was essentially the same as that in the lighter segment, where concentration apparently was not a factor, it seems doubtful that differences in concentration of oxygen administered to infants of multiple birth significantly altered the incidence-duration relation.

It is concluded that the graphs shown in Figure 5 present a good approximation of the relation between incidence and duration in oxygen unaffected by other factors, particularly for the first few days of exposure to oxygen. Thereafter, because of differences in health status, the rise in incidence may be less than it would be for a population of infants having a similar proportion of sick and well infants in all groups. *For most practical purposes it is noteworthy that for infants of single birth the maximal incidence rate appeared to be reached for exposures to oxygen of three days and less.*

The relation between incidence and duration in oxygen (infants of single birth) shown in Figure 4, where the raw data are presented without attempt to reduce the effect of any factors which might also have affected the incidence rate of RLF, appears to be slightly distorted in the initial portion of the curves, i. e., short durations of oxygen, owing to dissimilar birth weights in the various groups of infants. For a normal population of surviving premature infants of single birth (1500 gm. and less) the incidence could be expected to rise even more abruptly than is shown in Figure 4, i. e., similar to that indicated in Figure 5.

For infants of multiple birth, differences in birth weight do not appear to have

produced any distortion in the incidence-duration relation (Fig. 4), since the curves shown in Figure 5, which were compensated for birth weight, and the curves of Figure 4 do not differ appreciably.

Concentration of Oxygen and Incidence of RLF

To investigate the effect of concentration of oxygen on the incidence of RLF, the population of infants was separated into groups according to the average concentration of oxygen received by each infant for the entire length of exposure to oxygen. Eight groups were formed whose average concentration of oxygen differed by increments of 10%. The incidence of RLF, the average duration in oxygen, gestational age, and birth weight of the infants for these groups are shown in Table 10. The average gestational ages and birth weights for the groups were similar, and the individual values for the infants within each of the groups were

TABLE 10.—*Concentration of Oxygen*

Incidence of Cicatricial Retrolental Fibroplasia, Average Duration in Oxygen, Average Gestational Age, and Average Birth Weight of Infants Grouped According to Concentration of Oxygen

Av. Conc. O_2 (Each Infant's Total Exposure)	Incidence RLF		Av. Duration in O_2		Av. Gest. Age		Av. Birth Weight	
%	%		Days	Infants, No.	Wk.	Infants, No.	Gm.	Infants, No.
Single births								
80-89	0	(0/1)	1	1	--	0	1,225	1
70-79	0	(0/4)	4	4	31	2	1,281	4
60-69	8.3	(2/24)	16	24	31	14	1,187	24
50-59	10.3	(7/68)	16	68	31	56	1,280	68
40-49	5.6	(7/124)	8	124	31	105	1,221	124
30-39	6.2	(8/130)	8	130	31	103	1,231	130
21-29	0	(0/14)	3	14	32	9	1,230	14
Air	1.1	(1/93)	--	93	32	77	1,266	93
Average	5.5	(25/458)	8	458	31	366	1,240	458
Unknown conc.	21.4	(3/14)	2	14	31	14	1,315	14
Unknown other factors	--	--	--	0	--	92	--	0
All infants	5.9	(28/472)						
Multiple births								
80-89	0	(0/3)	3	3	34	3	1,125	3
70-79	100	(1/1)	11	1	33	1	1,450	1
60-69	33.3	(2/6)	5	6	32	2	1,246	6
50-59	23.1	(3/13)	16	13	31	8	1,235	13
40-49	29.2	(7/24)	4	24	33	21	1,307	24
30-39	12.9	(4/31)	7	31	32	27	1,270	31
21-29	11.1	(1/9)	5	9	33	7	1,302	9
Air	0	(0/19)	--	19	35	15	1,325	19
Average	17.0	(18/106)	5.5	106	33	84	1,280	106
Unknown conc.	12.5	(1/8)	2	8	32	6	1,195	8
Unknown other factors	--	--	--	0	--	24	--	0
All infants	16.7	(19/114)						

randomly distributed. However, the average duration in oxygen in the several groups differed appreciably.

In an effort to establish the true relation, therefore, between incidence and concentration, uninfluenced by differences in length of exposure, the infants were separated into two groups whose duration of exposure to oxygen was more and less than five days, and more and less than three days, for infants of single and multiple birth, respectively. These groups were subdivided into two others with respect to concentration, viz., those infants who received an average concentration of oxygen greater and less than 40% (Figs. 5A and 6A). The infants who did not receive supplementary oxygen were grouped separately. In this manner it was possible to obtain four groups of individuals, two of which had similar stays in oxygen, and a fifth group (labeled "air" in the Figures) which received no added oxygen.

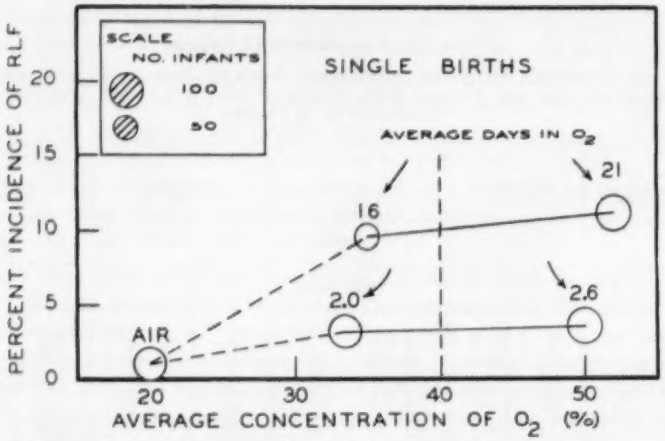


Fig. 6.—The relation between the incidence of cicatricial RLF and the average concentration of oxygen received by infants of single birth whose stay in oxygen was for similar periods of time.

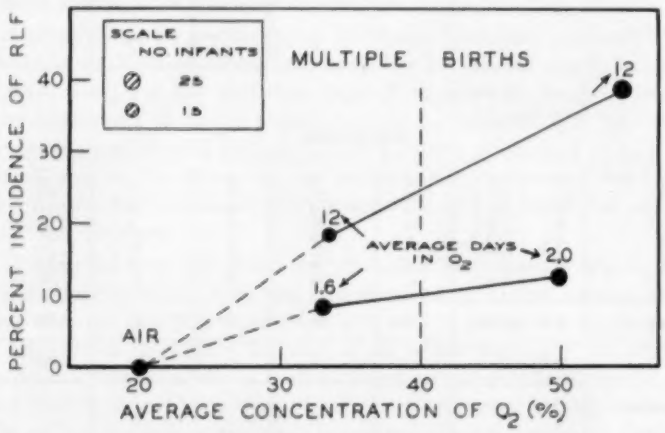


Fig. 7.—The relation between incidence of cicatricial RLF and average concentration of oxygen received by infants of multiple birth whose stay in oxygen was for similar periods of time.

The relation between incidence and average concentration of oxygen for these groups is shown in Figures 6 and 7. Differences in the average concentration of oxygen from approximately 35% to 50% given to infants of single birth, for either short periods (average of about two days) or periods of two to three weeks (average), appeared to have no appreciable effect on the incidence of RLF.

Similar differences in the average concentration (33% to 50%) given infants of multiple birth, who received oxygen for an average of two days or less, were likewise not accompanied by greatly increased incidence. The incidence did increase (doubly), however, with an increase in concentration of oxygen from 35% to 55% received by infants of multiple birth who were kept in oxygen for an average of 12 days.

Differences in the health status of the infants comprising the several groups were probably such as to decrease any dependence of incidence on concentration in the groups receiving oxygen for longer periods, i. e., 16 and 12 days (Figs. 6 and 7, respectively), since the group of infants exposed for several weeks at the lower average concentration, i. e., approximately 35%, probably contained a disproportionate number of infants who made poor progress and who were shown earlier in this report to exhibit a lower incidence of RLF for the same exposure to oxygen. In other words, these two groups of infants of single and multiple birth, respectively, consist chiefly of infants in the curtailed-oxygen group, who would, had they not been ill, have had a higher incidence of the disease—perhaps as high as that observed in the group (containing most of the routine-oxygen group infants) who received oxygen at average concentrations of over 50%.

It is concluded that for infants of single birth the incidence of RLF is relatively unaffected by differences in average concentration of oxygen in the range of 35% to 50%. For infants of multiple birth the incidence increased with concentration, but only to an appreciable degree in those infants who were exposed to oxygen over a relatively long period of time, i. e., of the order of two weeks.

It could be maintained that the use of the average concentration for the entire period each infant was in oxygen may lead to erroneous conclusions since the same average might be obtained from exposure of an infant to oxygen at two different concentrations throughout his stay in oxygen as would result from continuous exposure at the same concentration. Under these circumstances, any critical concentration above which RLF might occur with much greater frequency would be masked.

To eliminate this possibility, the degree to which the average concentration of oxygen fluctuated from day to day was determined for all the infants in each of the four groups shown in Figures 5A and 6A. These data are presented in Figures 7A and 8A for infants of single and multiple birth, respectively. The two longer lines of each graph correspond to the groups of infants represented by the two upper quadrants of Figure 5A (single births) and Figure 6A (multiple births), and the two shorter lines of each figure, to the groups of infants represented by the two lower quadrants of the corresponding figures.

Figure 7A shows that for each of the four groups of infants of single birth there was little variation in the concentration throughout the infants' stay in oxygen. The average concentration of oxygen from day to day was also approximately the same for all of the groups of infants of multiple birth (Fig. 8A), except for that one group whose average stay in oxygen was two days and whose average concentration was 50%. The Figure shows that in this group the concentration fell from an average value of approximately 57% on the first day to 37% on the third day. It is not possible to say whether the incidence in this group of infants would have been more or less than 11.8% had the concentration remained approximately at the 50% level for the three-day period.

With the above-mentioned exception, it may be concluded from the small daily fluctuations in average daily concentrations that the use of average concentrations of oxygen for the whole period of exposure is valid for determining the relation between incidence and concentration.

Gestational Age and Incidence of RLF

To investigate the effect of gestational age on the incidence of RLF, the population was separated into groups of infants having the same gestational ages (estimated from the date of the last menstrual period). The incidence of RLF, average duration in oxygen, concentration of oxygen, and birth weight for each of these groups are recorded in Tables 11 and 12 for infants of single and multiple birth, respectively. The relation between the incidence and gestational age is shown in Figure 8. For infants of single birth there is a slight inverse relation and for infants of multiple birth a definite inverse relation.

The average durations in oxygen and concentrations of oxygen for infants of single birth in these groups were similar (Table 11).* The average birth weights, however, increased with increase in gestational age up to 30 weeks, after which age they averaged approximately 1300 gm. Data have already been presented which suggest that the incidence of RLF is inversely related to birth weight (Fig. 5; see also Fig. 10). The correlation between birth weight and gestational age, therefore, makes it difficult to determine the extent to which the lower incidence observed in groups of infants having longer gestational ages may be due to gestational age alone.

For infants of multiple birth whose gestational age was less than 33 weeks the

* When those infants who received no additional oxygen were excluded from the groups, the incidence rate increased slightly, but the average duration in oxygen in the various groups still remained closely similar, as did the average concentration of oxygen. Hence, differences in distribution of infants in the groups shown in Table 11 who remained in an air environment did not mask any effect of gestational age on incidence.

TABLE 11.—*Gestational Age*

Incidence of Cicatricial Retrolental Fibroplasia, Average Duration in Oxygen, Average Concentration of Oxygen, and Average Birth Weight of Infants Grouped According to Gestational Age

	Single births					
Gestational Age	Incidence RLF	Av. Duration in O_2		Av. Conc. of O_2		Av. Birth Weight	
Wk.	%	Days	Infants, No.	%	Infants, No.	Gm.	Infants, No.
24 and less	6.2 (1/16)	5	16	38	14	1,160	16
25	0 (0/10)	17	10	38	10	1,070	10
26	25.0 (3/12)	17	12	43	10	1,154	12
27	15.4 (2/13)	6	13	42	13	1,066	13
28	5.0 (1/20)	9	20	40	20	1,187	20
29	8.7 (4/46)	9	46	45	46	1,186	46
30	7.4 (4/54)	8	54	37	53	1,292	54
31	3.7 (2/54)	6	54	39	54	1,208	54
32	6.7 (3/45)	6	45	39	43	1,308	45
33	4.5 (1/22)	6	22	38	21	1,247	22
34	10.0 (2/20)	5	20	36	20	1,286	20
35	6.2 (1/16)	8	16	38	15	1,271	16
36	0 (0/13)	7	13	35	13	1,370	13
37	20.0 (2/10)	7	10	41	9	1,254	10
38 and more	0 (0/29)	5	29	38	29	1,347	29
Average	6.8 (26/380)	7.4	380	39	370	1,250	380
Unknown gest. age	2.2 (2/92)	10	92	36	88	1,222	92
Unknown other factors	-- --	--	0	--	14	--	0
All infants	5.9 (28/472)						

TABLE 12.—*Gestational Age*

Incidence of Cicatricial Retrolental Fibroplasia, Average Duration in Oxygen, Average Concentration of Oxygen, and Average Birth Weight of Infants Grouped According to Gestational Age

Multiple births

Gestational Age	Incidence RLF		Av. Duration in O_2*		Av. Conc. of O_2*		Av. Birth Weight	
Wk.	%		Days	Infants, No.	%	Infants, No.	Gm.	Infants, No.
27	0	(0/2)	34	2	35	2	1,035	2
28	33.3	(1/3)	17	3	35	2	1,117	3
29	33.3	(2/6)	1.5	6	37	4	1,203	6
30	22.2	(2/9)	6	9	46	9	1,297	9
31	22.2	(2/9)	6	9	34	9	1,281	9
32	30.0	(3/10)	6	10	42	10	1,261	10
33	12.5	(2/16)	3	16	46	16	1,262	16
34	7.7	(1/13)	7	13	40	13	1,325	13
35	20.0	(1/5)	4	5	31	5	1,314	5
36	0	(0/7)	1	7	28	4	1,300	7
37	0	(0/4)	1	4	31	4	1,315	4
38 and more	0	(0/6)	2	6	28	6	1,390	6
Average	15.6	(14/90)	5.3	90	39	84	1,280	90
Unknown gest. age	20.8	(5/24)	8	24	38	22	1,275	24
Unknown other factors	--	--	--	0	--	8	--	0
All infants	16.7	(19/114)						

*Figures included those infants who did not receive oxygen.

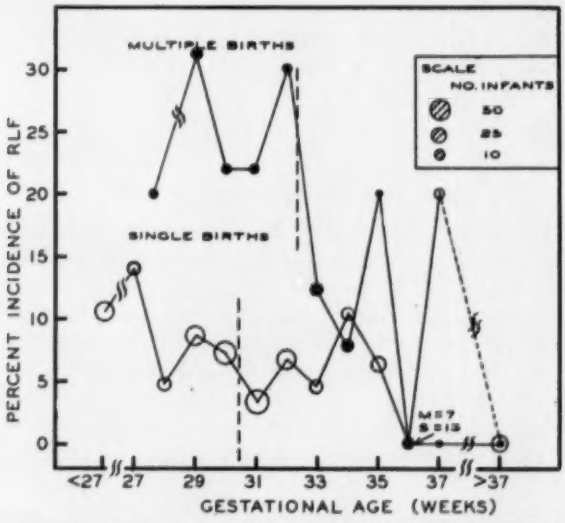


Fig. 8.—The relation between incidence of cicatricial RLF and gestational age of infants of single and of multiple birth.

average duration in oxygen was about 8 days, compared with about 4 days for infants who had gestational ages of 33 weeks and more. A similar difference in

duration of exposure to oxygen existed when those infants who had no exposure to additional oxygen were excluded from both groups.

However, the difference in average exposure to oxygen in these groups was caused largely by the effect of but six infants, who were in the group having the shorter gestational ages. If those infants whose stay in oxygen exceeded 10 days were eliminated from the two groups, then the average exposure to oxygen for the two groups would be much more similar, viz., 3 days for infants with the shorter gestational ages and 2½ days for the group with the longer gestational ages. The incidence rates still differed widely (23.4% and 6.4%, respectively). This suggests that not all of the difference in incidence (Fig. 8) can be accounted for by differences in duration of exposure to oxygen.

The possible effect of differences in concentration of oxygen on the incidence of RLF in infants of multiple birth who had different gestational ages is complicated by the fact that when the incidence of RLF is computed for groups of infants who had similar gestational ages as well as similar average concentrations of oxygen, dissimilarities again are found in duration of oxygen given these groups of infants. The data are shown in Figure 9A.

The factors of duration and concentration of oxygen for infants of multiple birth are so interrelated that, considering the number of infants available for analysis, it is not possible to determine independent effects with any degree of reliability.

Concerning the possible effect of differences in birth weight on the incidence of RLF in infants of multiple birth who had different gestational ages, Table 12 shows that, as for the infants of single birth, the average birth weight increased up to approximately the age of 30 weeks and then remained about the same. Because of the relation between gestational age and birth weight, the separate effect of the former also cannot readily be determined.

Gestational age does not appear to influence greatly the susceptibility of infants of single birth to RLF. Because of variations in duration of exposure to oxygen, concentration of oxygen, and birth weight, no definite conclusion can be drawn concerning the effect of gestational age on the susceptibility of infants of multiple birth to RLF.

Birth Weight and Incidence of RLF

To relate the incidence of RLF and birth weight of the infants, the population was divided into groups of infants whose birth weights fell within increments of 100 gm. The incidence of RLF, duration in oxygen, average concentration of oxygen, and gestational age for these groups are shown in Table 13. The relation between average birth weight and incidence of RLF for infants of single and multiple birth, separated in this manner, is shown in Figure 9. The graph shows that the incidence of RLF is, to a small extent, inversely related to birth weight. However, the data shown in the Table indicate that the length of stay in oxygen also varied inversely with birth weight, particularly in the instance of infants of single birth.

In the section of this paper which deals with duration in oxygen and incidence of RLF, the infant population was divided in such a way that the average stay in oxygen for the heavier and lighter segments of the population was approximately equal and then further divided into groups of infants who received oxygen for one to three days, four days and more, and also a group who received no addi-

TABLE 13.—*Birth Weight*

Incidence of Cicatricial Retrolental Fibroplasia, Average Duration in Oxygen, Average Concentration of Oxygen, and Average Gestational Age of Infants Grouped According to Birth Weight

Birth Weight	Incidence RLF	Av. Duration in O_2*		Av. Conc. of O_2*		Av. Gest. Age	
Gm.	%	Days	Infants, No.	%	Infants, No.	Weeks	Infants, No.
Single births							
600-699	0 (0/6)	26	6	32	6	27	5
700-799	0 (0/3)	33	3	37	3	25	2
800-899	6.2 (1/16)	14	16	44	16	28	10
900-1,000	15.2 (5/33)	13	33	39	33	29	20
1,001-1,099	8.0 (2/25)	11	25	45	24	29	25
1,100-1,199	9.8 (8/82)	8	82	40	79	31	65
1,200-1,299	6.2 (5/81)	7	81	40	81	31	64
1,300-1,399	3.8 (4/104)	7	104	38	100	32	85
1,400-1,500	2.4 (3/122)	5	122	37	116	32	104
Average	5.9 (28/472)	8	472	39	458	31	380
Unknown birth wt.	-- (0/0)	--	0	--	0	--	0
Unknown other factors	-- ---	--	0	--	14	--	92
Multiple births							
700-799	100 (2/2)	30	2	30	2	28	1
800-899	0 (0/1)	2	1	--	0	32	1
900-1,000	0 (0/2)	14	2	55	1	36	1
1,001-1,099	0 (0/8)	11	8	55	8	31	8
1,100-1,199	33.3 (5/15)	6	15	45	14	31	10
1,200-1,299	14.3 (4/28)	4	28	36	26	33	26
1,300-1,399	9.1 (3/33)	5	33	39	31	33	23
1,400-1,500	20.0 (5/25)	3	25	36	24	35	20
Average	16.7 (19/114)	5.5	114	40	106	33	90
Unknown birth wt.	-- (0/0)	--	0	--	0	--	0
Unknown other factors	-- ---	--	0	--	8	--	24

* Figures include those infants who did not receive oxygen.

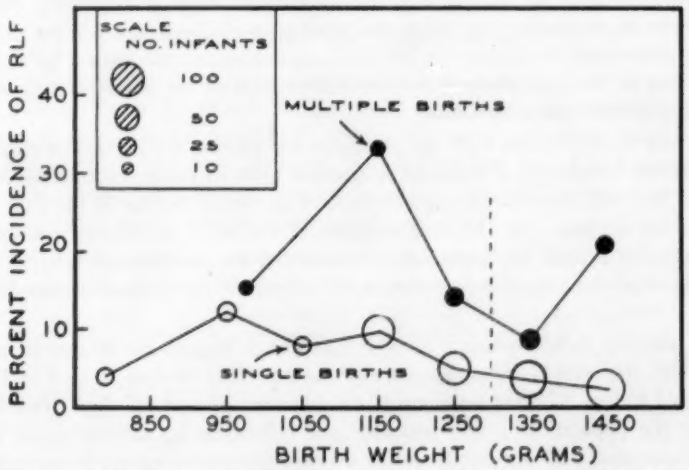


Fig. 9.—The relation between incidence of cicatricial RLF and birth weight of infants of single and of multiple birth.

tional oxygen (Figs. 3A and 4A). The relation between duration in oxygen and incidence for the heavier and lighter segments of the population was presented in Figure 5. The same data are now plotted to show the relation between incidence and birth weight (Fig. 10) for short and long durations in oxygen. It is apparent

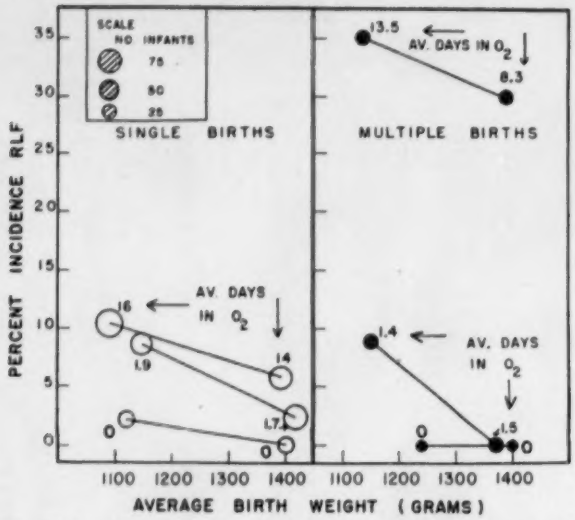


Fig. 10.—The relation between incidence of cicatricial RLF and average birth weight for groups of infants whose stay in oxygen was similar.

that except for those infants of multiple birth who received no added oxygen (and in whom there was no cicatricial RLF) the incidence of the disease varied inversely with the birth weight for infants of both single and multiple birth. The effect of duration in oxygen again becomes evident—this time from differences in the heights of the lines.

The relation between birth weight and incidence (Fig. 10) for infants of single birth would seem to be independent of any differences in susceptibility caused by differences in gestational age, since the average gestational age for the infants in all six groups used in making the plot was approximately the same (Table 9), and an analysis of the data showed that the distribution of the gestational ages within the weight groups was also similar.

It is not likely that the birth weight-incidence relation for infants of single birth was distorted because of differences in concentration of oxygen administered to the infants. Not only was the average concentration closely similar in the four groups given added oxygen (Fig. 10) but analyses of the effect of average concentration of oxygen for infants of single birth showed that the incidence of RLF is not appreciably dependent upon concentration of oxygen in the range between 35% and 50%.

The relation shown between incidence and birth weight for infants of multiple birth (Fig. 10) who received oxygen for short periods of time, i. e., 1 and 2 days (average 1.4 days [lighter segment of the population] and 1.5 days [heavier segment of the population]) was probably not influenced by differences in susceptibility associated with differences in the average gestational age or by concentration of oxygen given the two groups of infants (Table 14). The difference in gesta-

TABLE 14.—*Incidence of Cicatricial Retrolental Fibroplasia, Average Gestational Age, and Average Concentration of Oxygen of Infants Grouped According to Birth Weight and Duration in Oxygen*

Multiple Births				
Birth Weight Grouping	Oxygen Grouping	Incidence RLF, %	Av. Gestational Age, Wk.	Av. Concentration of O_2, %
1,300 gm. and more	1 and 2 days	0	34	37
1,299 gm. and less	1 and 2 days	9.1	32	44
1,300 gm. and more	3 days and more	30	33	46
1,299 gm. and less	3 days and more	35	32	47

tional age was small (one week), and differences in concentration of oxygen given infants of multiple birth for short durations was shown to be without effect on incidence (Fig. 7).

In the instance of infants of multiple birth who received oxygen for the longer periods of time, i. e., 3 days or more (average 13.5 days [lighter segment of the population] and 8.3 days [heavier segment of the population]), both the average gestational age and concentration of oxygen given the two groups were closely similar. So far as gestational age and average concentration of oxygen are concerned, no distortion of the incidence-birth weight relation would be expected. The duration in oxygen, however, was greater for the infants in the lighter segment of the population. This would have the effect of augmenting the apparent dependence of incidence upon birth weight.

Differences in the health status of infants of multiple birth, as well as of single birth, are in the direction to have decreased the dependence of incidence upon the birth weight (Fig. 9), for it has been shown that the lighter infants, who frequently had the longer stays in oxygen and who more often were ill, appeared to be less susceptible to a given duration in oxygen.

The above analyses suggest that there is probably an inverse relation between birth weight and incidence, for infants of single birth, and probably also for infants of multiple birth, but in the latter instance other factors are so interrelated that this conclusion remains uncertain.

Sex of the Infant and Incidence of RLF

The data thus far have been separated without regard to the sex and race of the infant. Table 15 shows the incidence of active and cicatricial RLF of male and female infants separately. Figure 11 shows the incidence of RLF for infants of single and multiple birth divided according to sex and grouped also according to similar periods of time spent in an oxygen-enriched environment, in a manner analogous to that used in preparing Figure 4.

TABLE 15.—*Incidence of Active and Cicatricial Retrolental Fibroplasia According to Sex*

Sex	Single Births		Multiple Births	
	Active RLF, %	Cicatricial RLF, %	Active RLF, %	Cicatricial RLF, %
Males	36 (72/201)	7.5 (15/201)	42 (20/48)	14.6 (7/48)
Females	35 (94/271)	4.8 (13/271)	45 (30/66)	18.2 (12/66)
Total	35 (166/472)	5.9 (28/472)	44 (50/114)	16.7 (19/114)

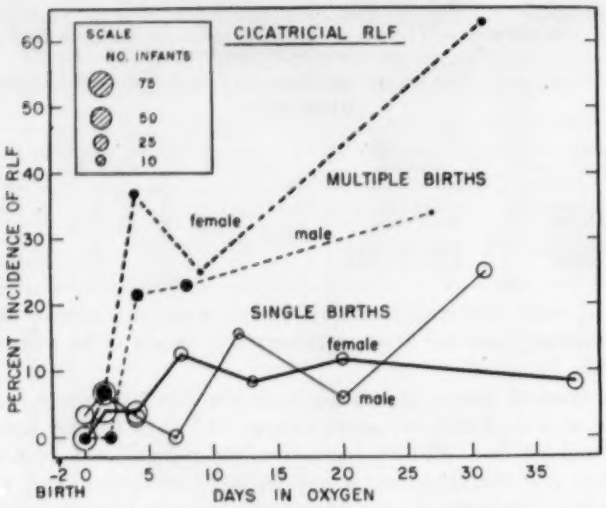


Fig. 11.—The relation between incidence of cicatricial RLF and number of days male and female infants were in oxygen after admission to the study.

There is no significant difference in the incidence of active RLF, or of cicatricial RLF, between male and female infants of either single or multiple birth. As stated previously, the incidence of RLF in the whole population differed significantly ($P<0.01$) between infants of single and multiple birth, the incidence being higher in the latter instance (Fig. 4). The difference in incidence between female infants of single and of multiple birth also is significant at the 1% level. The difference in incidence for male infants of single and multiple birth is not significant, although the rate for the male infants of single birth is consistently lower for exposures of oxygen beyond three days (Fig. 11).

The average gestational ages for male and female infants in the groups used in preparing Figure 11 were approximately the same and of closely similar distribution. The average birth weight for female infants was 1255 and 1287 gm. for infants of single and multiple birth, respectively, compared with average birth weights of male infants of 1319 and 1275 gm. for the same respective groups.

It is concluded that there is no appreciable difference in the susceptibility between male and female infants of either single or multiple birth.

Race and Incidence of RLF

There was no significant difference between the incidence of cicatricial RLF in white and in nonwhite infants of either single or multiple birth, although there was a slightly greater incidence in infants of both categories for the white infants (Table 16). The Table shows that the average number of days in oxygen of the white and nonwhite infants in both groups was similar.

Because of the polyphyletic nature of the study population, particularly among the "white" infants, *it is not possible to decide with any degree of certainty the comparative susceptibility of different races to RLF.*

Type of Incubator and Incidence of RLF

The incidence of both active and cicatricial RLF in infants grouped according to the type of incubator used is shown for infants of single and multiple birth in

TABLE 16.—*Incidence of Cicatricial Retrolental Fibroplasia and Average Days in Oxygen in White and Nonwhite Infants*

Race	Single Births		Multiple Births	
	Incidence RLF, %	Av. Days in O_2	Incidence RLF, %	Av. Days in O_2
White	7.1 (15/212)	7.6	20.4 (9/44)	4.8
Nonwhite	5.0 (13/260)	8.2	14.3 (10/70)	6.2
Total	5.9 (28/472)	7.9	16.7 (19/114)	5.7

Table 17. (When an infant was kept in more than one type of incubator, only the

TABLE 17.—*Incidence of Active and Cicatricial Retrolental Fibroplasia According to Type of Incubator Used* *

Type of Incubator	Single Births		Multiple Births	
	Active RLF, %	Cicatricial RLF, %	Active RLF, %	Cicatricial RLF, %
Isolette	35 (121/347)	5.8 (20/347)	45 (30/67)	15 (10/67)
Gordon Armstrong	38 (38/101)	5.9 (6/101)	51 (20/39)	23 (9/39)
Hess bed	24 (5/21)	9.5 (2/21)	0 (0/8)	0 (0/8)
Crib	100 (2/2)	0 (0/2)	0 (0/0)	0 (0/0)
Unknown	0 (0/1)	0 (0/1)	0 (0/0)	0 (0/0)
Total	35 (166/472)	5.9 (28/472)	44 (50/114)	17 (19/114)

* The first type of incubator in which the infant was placed is used for this tabulation.

data from the first type were used to prepare the Table.) *The incidence rates of both the active and the cicatricial form of the disease did not appear to be dependent upon the type of incubator employed.*

Miscellaneous Factors and Incidence of RLF

To determine the possible association of factors pertaining to the infant and mother which have not heretofore been considered in the development of RLF infants were grouped according to whether their eyes remained normal (NNN), developed an active stage of RLF and then became normal (NAN), or developed cicatricial RLF (NAC). The frequency of administration of various medications and dietary adjuncts to the infants and the complications of pregnancy in the mother were determined and are shown in Table 18. The average age of the mother for each of the groups of infants is also shown.

The frequency of transfusions and infusions was slightly higher in the groups of single and multiple infants who developed cicatricial RLF. This observation is consistent with the fact that the group of infants who developed cicatricial RLF contained a disproportionately greater number of infants of lower birth weight.

The frequency of administration of chemotherapeutic agents, antibiotics, other medications, and dietary adjuncts to the infants, as well as the frequency of complications of pregnancy in the mothers, was closely similar in all three groups of infants of both single and multiple birth.

The average age of the mothers in these groups likewise was not significantly different.

No factor, therefore, other than that associated with exposure of the infant to an oxygen-enriched environment, was found to be positively associated with the incidence of either active or cicatricial RLF.

TABLE 18.—*Miscellaneous Factors Associated with Infant and Mother, Grouped According to Ocular Findings*

Factors	Single Births			Multiple Births		
	NNN*	NAN*	NAC*	NNN*	NAN*	NAC*
	Per Cent of Total Population			Per Cent of Total Population		
Transfusions	8.5 (26/306)	2.9 (4/138)	11 (3/28)	6 (4/64)	0 (0/31)	11 (2/19)
Infusions	27 (83/306)	43 (60/138)	68 (19/28)	36 (23/64)	32 (10/31)	32 (6/19)
Chemotherapy & antibiotics	71 (216/306)	86 (118/138)	86 (24/28)	67 (43/64)	61 (19/31)	5 (1/19)
Other medications	33 (102/306)	43 (60/138)	39 (11/28)	27 (17/64)	29 (9/31)	37 (7/19)
Dietary adjuncts	65 (200/306)	55 (76/138)	64 (18/28)	72 (46/64)	68 (21/31)	58 (11/19)
Complications of pregnancy	40 (121/306)	46 (64/138)	50 (14/28)	45 (29/64)	32 (10/31)	58 (11/19)
Av. age of mother, yr.	25	27	28	26	24	26

*NNN, infants whose eyes showed no signs of RLF; NAN, infants whose eyes showed active stages of RLF and then regressed to normal; NAC, infants whose eyes showed active stages of RLF and developed cicatricial RLF.

Severity of RLF

Previous sections of this report have been concerned chiefly with the incidence of RLF as affected by various environmental and congenital factors, without regard to severity of the disease. The relations between severity (graded A through E) of the disease for each eye and time in oxygen, concentration of oxygen, gestational age, and birth weight of infants of single and multiple births are shown in Figures 12 through 15, respectively.

The Figures show that the severity of RLF in infants of both single and multiple birth was not dependent upon the number of days the infant was exposed to oxygen, the average concentration of oxygen employed, gestational age, or birth weight. However, proportionately more infants of multiple than of single birth exhibited the severer forms of the disease, particularly in the instance of infants who received oxygen for only a few days.

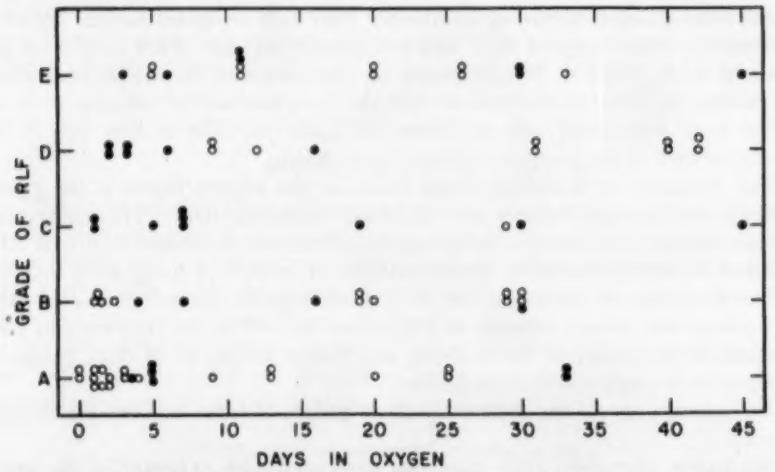


Fig. 12.—The severity of cicatricial RLF in infants arranged according to number of days in oxygen. Each open circle represents an eye of an infant of single birth; each filled circle represents an eye of an infant of multiple birth.

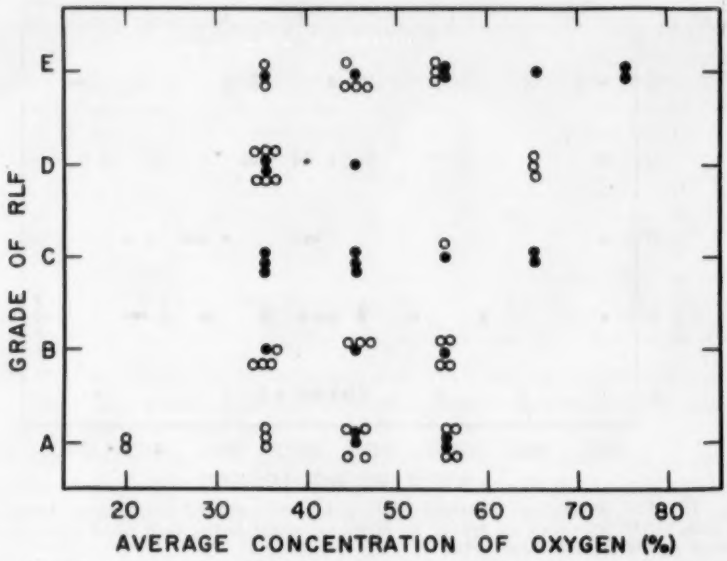


Fig. 13.—The severity of cicatricial RLF in infants arranged according to average concentration of oxygen received. Each open circle represents an eye of an infant of single birth; each filled circle represents an eye of an infant of multiple birth.

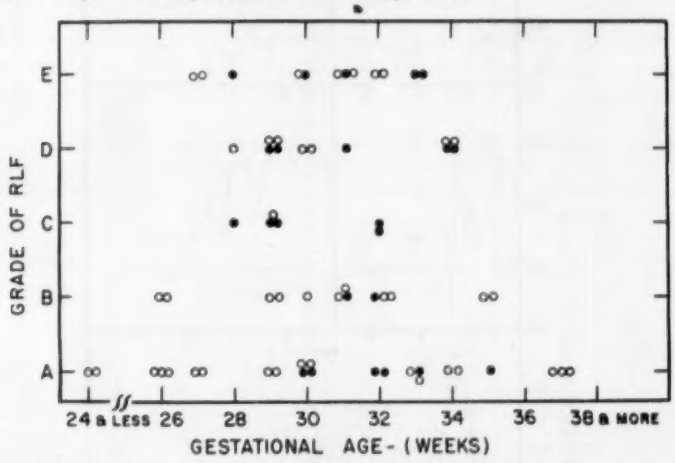


Fig. 14.—The severity of cicatricial RLF in infants arranged according to gestational age. Each open circle represents an eye of an infant of single birth; each filled circle represents an eye of an infant of multiple birth.

That severity of the disease is not dependent on either duration in oxygen or concentration employed can be seen from Figure 16, which presents variation in severity of the disease in infants who were exposed to oxygen for short, intermediate, and relatively long periods at concentrations greater and less than 40%. In two instances (of a total of six similar ones) not only was the average concentration below 40% but from repeated analyses it appears that the concentration never exceeded 40%. The data presented in the lower part of the Figure show that the severity of the disease varies widely with closely similar experiences in

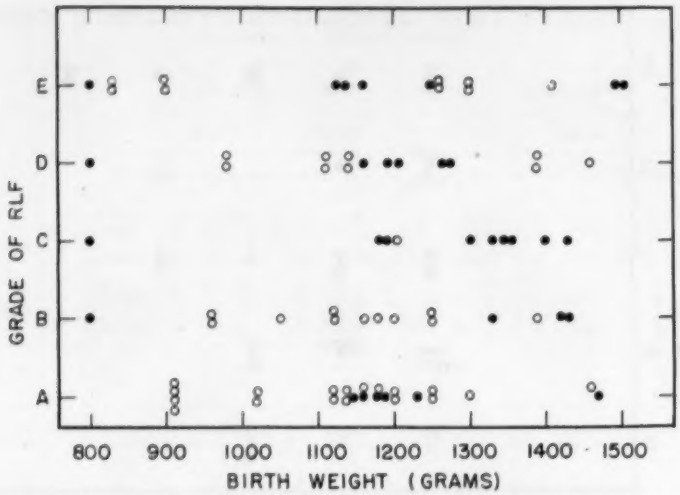


Fig. 15.—The severity of cicatricial RLF in infants arranged according to birth weight. Each open circle represents an eye of an infant of single birth; each filled circle represents an eye of an infant of multiple birth.

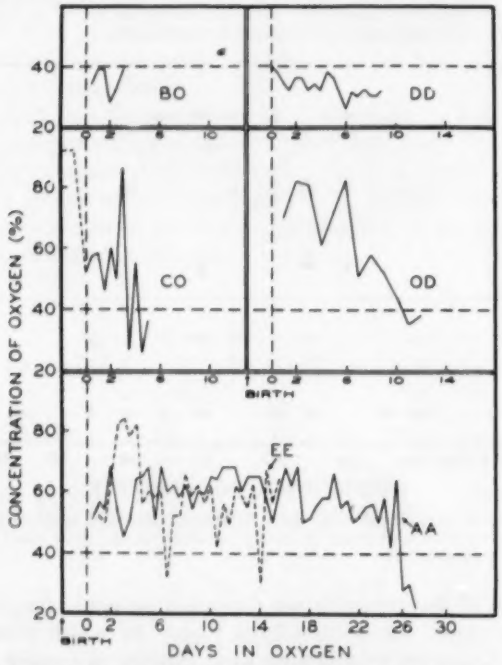


Fig. 16.—Several typical cases of RLF showing different experiences with respect to concentration of oxygen received and duration in oxygen.

oxygen. Both eyes of one infant developed RLF, Grade A, while both eyes of another developed Grade E.

The relative severity of cicatricial RLF in white and nonwhite infants, of

single and multiple birth, separated according to sex, is shown in Table 19. As with

TABLE 19.—*Severity of Cicatricial Retrolental Fibroplasia According to Sex and Race*

Race	Single Births		Multiple Births	
	Males	Females	Males	Females
White	AA BA AA BB DD DD EE EE	AA OA BO BB BC DD EE	BO ?C CC	AO AA BD DD EE EE
Nonwhite	AA AA AA OB OB EO EE	AA AO AO BB OD DD	AO CO BC ED	AA BC CC DD OE CE

incidence of the disease, the severity was not dependent upon either race or sex. Neither was it dependent upon the geographical location of the hospital nurseries where the infants were cared for.

The data indicate that the severity of cicatricial RLF is unrelated to duration of exposure to oxygen, concentration of oxygen, gestational age, birth weight, race, sex, and geographical location of the hospital nursery. The possibility that two or more of these factors might have acted oppositely to influence severity, however, cannot be excluded by the available data.

Spontaneous Regression of RLF

The percentage of infants whose eyes showed active stages of RLF but did not develop the cicatricial form of the disease, i. e., those infants whose eyes regressed to normal spontaneously, is shown in Table 20. The infants are grouped according

TABLE 20.—*Rate of Regression of Active Stages of Retrolental Fibroplasia According to Oxygen Group and Single and Multiple Birth*

RLF Maximal Active Stage (Poorer Eye)	Group		Birth	
	Routine O_2, % Regression	Curtailed O_2, % Regression	Single, % Regression	Multiple, % Regression
1	78.6 (11/14)	94.3 (99/105)	92.1 (82/89)	93.3 (28/30)
2	83.3 (10/12)	84.6 (33/39)	91.3 (42/46)	20.0 (1/5)
3	57.1 (4/7)	47.6 (10/21)	60.0 (12/20)	25.0 (2/8)
4	25.0 (1/4)	11.1 (1/9)	25.0 (2/8)	0 (0/5)
5	0 (0/1)	0 (0/4)	0 (0/3)	0 (0/2)
Total 1-5	68.4 (26/38)	80.3 (143/178)	83.1 (138/166)	62.0 (31/50)
Infants with no eye changes	... 15	... 355	... 306	... 64
Total no. infants	... 53	... 533	... 472	... 114

to protocol, i. e., by "oxygen assignment," and also according to single and multiple birth. There was no significant difference in the rate of spontaneous regression in the infants in the routine- and curtailed-oxygen groups. The rate of spontaneous regression for infants of multiple birth, however, was significantly less than that for infants of single birth, except from Stage 1. This difference in rate of regression accounts, in considerable part, for the higher incidence of the cicatricial form of the disease in infants of multiple birth. The large proportion of infants of single birth whose eyes developed active Stages 1 and 2,

but which did not develop cicatricial RLF, indicates a favorable prognosis during the early stages of the disease.

Age of Infants at Onset of RLF

Relation to Gestational Age and Birth Weight

Whether the onset of RLF is associated with some critical period in the development of the premature infant is not known. In an attempt to answer this question, the age at onset of RLF was correlated with the gestational age and birth weight of the infant (Figs. 17 and 18). The line in Figure 17 is drawn so that all of the

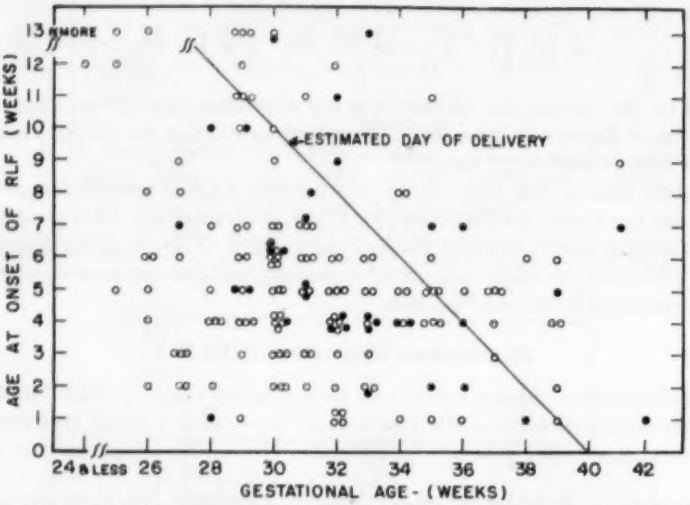


Fig. 17.—The relation between age at onset of active RLF and gestational age of the infant. Open circles represent infants of single birth; filled circles represent infants of multiple birth.

points on it correspond to an estimated delivery time of 40 weeks.

The age at onset of the disease appeared to be unrelated to either gestational age or birth weight. The correlation coefficients for gestational age, for infants of single and multiple birth, were −0.26 and −0.09, and for birth weight, −0.06 and −0.12.

Approximately one-fifth of all the premature infants who developed recognizable signs of RLF did so after the estimated time of delivery for full-term birth. With few exceptions, the eyes of these infants had been examined at least once before and had been found to have normal fundi at the estimated 40 weeks. Thus, the first signs of the disease which are visible ophthalmoscopically sometimes do not appear until the eyes have reached the state of development corresponding to full term.

The lack of apparent dependence of age at onset of the disease on gestational age suggests that the visible retinal vascular proliferative response to oxygen is not critically dependent upon the developmental state of the eye up to term, at least for infants whose birth weight is 1500 gm. or less.

Relation of Age at Onset to Time at Removal from Oxygen

The age of the infants at the time of onset of RLF and the number of days they were kept in an oxygen-enriched environment are shown for infants of both single and multiple birth in Figure 19. There was no apparent difference in the

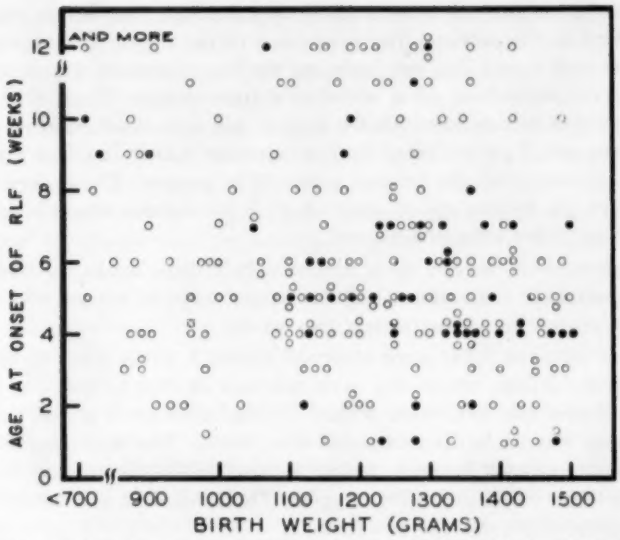


Fig. 18.—The relation between age of onset of active RLF and birth weight of the infant. Open circles represent infants of single birth; filled circles represent infants of multiple birth. (One infant of multiple birth who developed cicatricial RLF was not seen during the active stages of the disease and thus is not included.)

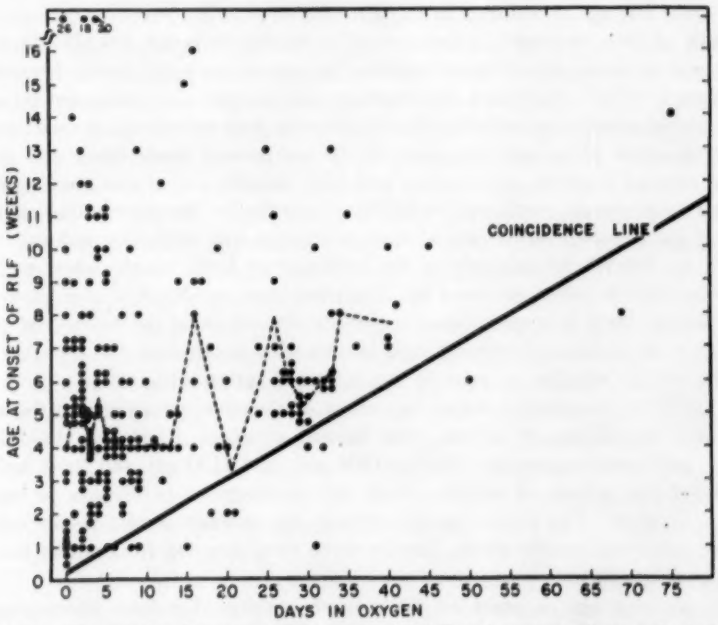


Fig. 19.—The relation between age at onset of RLF and number of days the infant was given oxygen after admission to the study. The broken line represents the mean age at onset for infants whose stays in oxygen were the same or similar. Filled circles represent infants of either single or of multiple birth.

relation of age at onset and length of time in oxygen between those infants whose eyes later regressed to normal and those who developed the cicatricial form of the

disease. In view of this, all infants whose eyes developed any active stage of RLF are considered in this section. The solid line † of the Figure (a co-incidence line) is drawn in such a way that any point on the line represents the situation where age at onset corresponds to age at withdrawal from oxygen. Thus, all circles above the line represent infants in which the disease was first observed after withdrawal from oxygen, and all circles below the line represent infants in which the first signs of RLF were seen while the infants were still in oxygen. The broken line of the Figure shows the median age at onset of RLF for infants whose lengths of stay in oxygen were either alike or nearly so.

For exposures of oxygen up to approximately three weeks the median age at onset was just over four weeks, and for longer stays in oxygen the median age at onset increased by approximately two weeks.

The first signs of RLF were observed within 3 weeks after birth for about one-third of the infants whose stay in oxygen was 10 days or less, whereas the onset of the disease was first noted within 3 weeks after birth in less than 10% of infants whose stay in oxygen exceeded this period. The data suggest that there may be a direct relation between the age at onset of the disease and the length of time of exposure of the infant to oxygen. The onset does not usually occur immediately after withdrawal from oxygen.

The correlation coefficient between the age at onset and length of time of exposure to oxygen is $+0.225$ ($P<0.01$).

Silverman and co-workers [11] similarly found a positive correlation between age at onset and age at cessation of oxygen (for all practical purposes the equivalent of length of time in oxygen), the correlation coefficient being $+0.375$. However, they found an inverse correlation between the age at onset and the birth weight of the infant (-0.51), and when the effect of birth weight was eliminated by means of the partial-correlation technique the association between the age at onset and the age at cessation of oxygen disappeared. In the present study there was also an inverse relation between age at onset and birth weight, but it was much less pronounced (correlation coefficient, -0.10). Accordingly, the correlation coefficient between age at onset and length of time in oxygen was negligibly reduced (from $+0.225$ to $+0.20$) by minimizing the influence of birth weight when the same partial-correlation technique used by Silverman and associates was employed.

However, there is a question as to the validity of using the method of partial correlation in problems involving data of this kind because of the apparent nonlinearity of the relation as seen in the scatter diagram (Fig. 19).

In an effort to visualize better any effect of duration in oxygen on the age at onset, the population of infants who showed signs of RLF was divided into heavier and lighter segments (1101 to 1500 gm., and 1100 gm. and less) and then subdivided into groups of infants whose stay in oxygen was 19 days or less and 20 days or more. The values for the average age at onset of the disease, time in oxygen, and birth weight of the infants were then obtained for each of the populations (Fig. 10A).

The average age at onset of RLF in the group of infants comprising the heavier segment of the population whose stay in oxygen was less than 20 days (average of 3.8 days) was 5.3 weeks, and the average birth weight of the infants

† The line does not pass through zero on the Y-axis because the age of the infants is 2 days greater than the indicated duration of exposure to oxygen, since the infants were not admitted to the study until 48 hours of age.

in this group was 1300 gm. The average age at onset in the group of infants whose stay in oxygen was 20 days or more (average of 30 days) was 6.8 weeks, and the average birth weight was 1290 gm. The difference in age at onset between the two groups is significant at the 0.01 level.

The age at onset for the groups of infants in the lighter segment of the population (lower two quadrants) was also greater in that group of infants whose stay in oxygen was the longer, and the average birth weights were similar. However, the difference is not significant. These observations suggest that the age at onset of the disease may be prolonged by the length of time the infant is kept in oxygen.

That the average age at onset of RLF was not dependent upon birth weight follows from the observation that the average age at onset was the same for those infants whose stay in oxygen was 20 days or more in both the lighter (910 gm.) and heavier (1290 gm.) groups of infants.

Similarly, there was no significant difference in the age at onset of RLF in the two groups of infants who were kept in oxygen for 19 days or less, despite differences in average birth weight of 360 gm. The average age at onset for the lighter and heavier groups was 6.2 and 5.3 weeks, respectively ($P=0.3$).

It is thus concluded that the age at onset of the disease, particularly for long exposures to oxygen, exceeding several weeks, is dependent upon the length of stay in oxygen but is not influenced by either birth weight or gestational age.

Weight Gain of Infants and RLF

The average weight gain of infants during the first 40 days of life grouped according to whether the eyes remained normal (NNN), developed active RLF and later regressed to normal (NAN), or developed cicatricial RLF (NAC) is shown in the upper portion of Table 21 for infants of single and multiple birth. The average birth weight for each of these groups is also shown. The same information for the infants classified according to the oxygen group to which they were assigned by protocol is given in the lower portion of the Table. The per cent of the population of infants of single birth, in each of these categories, divided into groups of infants whose average weight gain varied by increments of approximately 250 gm., is shown in Figure 11A.

For infants of single birth there was an inverse relation between weight gain and extent of ocular involvement. The greatest average gain in weight was made by those infants whose eyes remained normal, the next greatest by the group of infants who developed the active disease but whose eyes regressed to normal, and the least weight gain was made by the group of infants who developed cicatricial RLF. Possibly some of the differences in weight gain in these infants could be accounted for on the basis of the difference in average birth weight, since the average birth weight in the NNN group was over a hundred grams greater than in the NAC group.

In view of the positive association between incidence of RLF and the use of oxygen, and the fact that the sicker segment of the population received longer exposure to oxygen on the average, it is not unexpected that the average weight gain for infants who developed cicatricial RLF was less than that for those whose eyes remained normal.

For infants of multiple birth both the average weight gain and the average birth weight were similar in all three groups.

TABLE 21.—*Average Weight Gain of Infants During First Forty Days of Life, Classified According to Ocular Findings and Oxygen Grouping*

	NNN*			NAN*			NAC*			Total		
	Gain, Gm.	Av. Birth Weight, Gm.	Infants, No.	Gain, Gm.	Av. Birth Weight, Gm.	Infants, No.	Gain, Gm.	Av. Birth Weight, Gm.	Infants, No.	Gain, Gm.	Av. Birth Weight, Gm.	Infants, No.
Single births	516	1,269	282†	430	1,205	138	389	1,163	28	481	1,240	448†
Multiple births	477	1,287	59†	475	1,266	29†	510	1,232	19	482	1,272	107†

	Routine Oxygen Group			Curtailed Oxygen Group		
	Gain, Gm.	Av. Birth Weight, Gm.	Infants, No.	Gain, Gm.	Av. Birth Weight, Gm.	Infants, No.
Single births	489	1,233	45†	479	1,240	403†
Multiple births	485	1,287	6	481	1,270	101†

* NNN, infants whose eyes showed no signs of RLF; NAN, infants whose eyes showed active stages of RLF and then regressed to normal; NAC, infants whose eyes showed active stages of RLF and developed cicatricial RLF.

† Discrepancies between number of infants cited and those listed for each category in other tables result from some infants being discharged from hospital nurseries prior to 40 days of age.

Why the average weight gain for infants of multiple birth was not less in the group who developed cicatricial RLF than for those whose eyes remained normal is not apparent, since this group, like the analagous one for infants of single birth, also presumably represented the sicker segment of the population. However, the magnitude of this expected effect is not large and the number of infants in the groups under consideration is too small for adequate statistical evaluation.

From the lower portion of Table 21 it may be seen that, for infants of both single and multiple birth, the average weight gain in the routine-oxygen group was essentially the same as that in the curtailed-oxygen group.

Humidity and RLF

The per cent humidity in the incubator was recorded daily in a number of hospital nurseries. Considering the inaccuracies of the method of measurement, and the few data available on this subject, no conclusions could be drawn concerning the possible association of differences of humidity and the incidence of RLF.

Comment

The number of infants who would have had to have been in the routine-oxygen group for various possible total numbers of infants in the study, based on the incidence rates of RLF as observed in the two oxygen groups, is shown by the broken line of Figure 1. On the basis of these rates and for the confidence level selected (0.01), only about 30 instead of 53 of the 586 infants followed sufficiently long to make a diagnosis would have had to have been in the routine-oxygen group. As a result, the case for the association between oxygen and RLF was slightly overproved.

At the time the Cooperative Study was begun it was common practice in most of the hospital nurseries to administer oxygen in a manner similar to that prescribed for the routine-oxygen group. Considering the much higher incidence of RLF observed in the routine-oxygen than in the curtailed-oxygen group, it is worth noting that, by adhering to a design in which less than 10% of the total number of infants was assigned to the routine-oxygen group, many infants were undoubtedly saved from blindness.

The data collected during the Cooperative Study have shown conclusively that the incidence of RLF is positively associated with the length of time the premature infant is kept in an oxygen-enriched environment. The incidence of cicatricial RLF was not appreciably influenced by differences in the average concentration of oxygen received within the range of approximately 30% to 50%, with the exception of a relatively small group of infants of multiple birth who received oxygen for an average of almost two weeks, in which event the incidence increased with increased concentration.

The recommendation that the concentration of oxygen used in the care of the premature infant should not exceed 40% has been given widespread publicity by various medical and lay agencies. This advice is based largely on the experience at the Bellevue Hospital, New York,[12] where no cases of cicatricial RLF were observed in 81 infants (birth weights 1000 to 1850 gm.) who received "oxygen only at times of clinical need, and then for as brief periods as possible at concentrations less than 40 per cent."

In view of the evidence from the Cooperative Study that the incidence of RLF

is not greatly influenced, generally speaking, by differences in the average concentration of oxygen in the range of 30% to 50%, and that the disease has been observed to develop in a number of infants (6 infants out of a total of 104)‡ who received added oxygen but never in concentrations in excess of 40%, the absence of cicatricial RLF observed by the Bellevue Hospital investigators may have resulted because the duration of exposure to oxygen was short, rather than because the concentration was kept under 40%. In any event, it is not the present intent to suggest that concentrations of oxygen of 40% or less might not in fact involve less risk of RLF, on the average, than concentrations of 50% or less, for instance, but that there seems to be no particular basis for selecting the value of 40%.§ The results of this Cooperative Study would suggest that the basic policy to be adopted in prescribing oxygen should be to keep the premature infant in oxygen for as short a period as possible consistent with the clinical indications of anoxia, regardless of the concentration of oxygen employed. Recommendations based on this reasoning obviate the danger that some physicians may gain the impression that if the concentration is kept under some particular value the need for restricting the duration in oxygen is lessened. By following the above recommendations, and only in this manner, apparently, the over-all incidence of RLF can be kept at a minimum compatible with the survival of the infant.

The difference in the conclusion reached by Silverman and co-workers [11] and that arrived at in the present study, with regard to the dependence of age at onset of RLF on the length of stay in oxygen, is dependent upon differences in the results relating age at onset of RLF to birth weight. The results of Silverman and co-workers agree with those obtained in this study in showing that the age at onset was positively related to the length of time in oxygen. However, Silverman and his associates found that age at onset was inversely related to birth weight, while no substantial relation of this kind could be shown in the present study. For his data when the effect of birth weight was minimized statistically the resulting correlation between age at onset and age at cessation of oxygen disappeared,‖ whereas for the data obtained in the Cooperative Study the same mathematical treatment leaves the relationship between the age at onset and the length of time in oxygen substantially unaffected. It does not necessarily follow that the age at onset of RLF for the infants studied by Silverman and his associates, however, was not in fact influenced by the length of time in oxygen, since correlation with birth weight may merely have been a happenstance. The statistical treatment only shows that the relation was not significant.

The question of whether RLF is initiated by the procedure of rapid withdrawal from oxygen has been discussed often in the ophthalmic literature. Because the first signs of RLF are seen usually after the infants are removed from oxygen,

‡ For infants of single birth, 3 cases out of 78 infants. For infants of multiple birth, 3 cases out of 26 infants. The average stay in oxygen for these respective groups was 5 and 3 days, and for the 3 infants of single birth and 3 of multiple birth who developed the disease the average stay was 10 and 8 days, respectively.

§ Thus Patz (personal communication to the authors; to be published) has also demonstrated that oxygen when given at concentrations of 40% produces retinal changes. He has shown that one-fourth of the animals exposed to oxygen at concentrations of 35% to 40% for 10 days developed typical RLF lesions and concludes that duration of exposure to oxygen is the single most critical factor in the production of experimental RLF.

‖ Minimizing the effect of duration in oxygen did not appreciably affect the correlation between the age at onset and the birth weight.

and because, at least in the present study, the age at onset of the disease was apparently greater for infants who were exposed to oxygen for longer periods, it does not necessarily follow that RLF results from any procedure associated with withdrawal per se. Actually, although relatively infrequent, some cases of RLF in both this study and in numerous previous ones were observed to occur before removal from oxygen. Moreover, the incidence of the disease for infants who had relatively rapid withdrawal, but who had one or two days of oxygen, was less than that in the group of infants who received oxygen for a much longer period of time and who were withdrawn more slowly. These observations indicate that the exposure of an infant to oxygen, not the act of withdrawal, is the initiating factor which eventually leads to the pathologic changes in the retina which culminate in RLF, even though the time of appearance of the first changes may be delayed while the infant is kept in oxygen. The possible mechanisms associating retinal changes while in oxygen and pathogenesis of RLF have been discussed recently by Patz.[13]

The explanation for the significantly higher incidence of cicatricial RLF in infants of multiple birth is not apparent.

The observed phenomenon can only in part be accounted for by differences in susceptibility of retinal vessels to injury by oxygen, as has been pointed out earlier in this report. The higher rate of spontaneous regression observed for infants of single birth compared with that of multiple birth suggests that once the pathologic process has started there is a greater tendency for it to continue in infants of multiple birth until irreversible damage has occurred.

Retinal vessels of infants of multiple birth may possibly be less well developed for a given gestational age than those of single birth. However, after taking other factors into account, the incidence of RLF was found to be only relatively slightly dependent upon gestational age. It follows that, even though retinal development might lag in infants of multiple birth by several weeks, the effect on incidence would not likely be sufficient to account for the difference in incidence rates observed between infants of single and multiple birth. Furthermore, examination of histological specimens of eyes of infants of single and multiple birth of equal gestational age did not reveal any conspicuous difference in the relative state of development of the retinal vessels. India ink injections before sectioning might yield more definitive results.¶

It is possible also that the degree of saturation of oxygen in the blood of twin fetuses, say, might be less than that in single fetuses. Were this the case, exposure of an infant of multiple birth to an oxygen-enriched environment would result in a relatively greater difference in blood oxygen-saturation before and after birth, and thus possibly constitute a greater insult to the retinal vessels.

These explanations are merely conjectures, and *it is concluded that the true reason for differences in incidence between infants of single and multiple birth remains unknown.*

The average weight gain of all the infants of the study population during the first 40 days of life was approximately 480 gm., and the average birth weight was approximately 1250 gm. The weight gain was about 80 gm. lower than that cited

¶ Michaelson[14] found no clear-cut difference in developmental state between two cases of twins and infants of single birth using this technique, but noted that the India ink leaked out of the retinal vessels of eyes of twins to a greater extent than those of single birth, suggesting differences in the vessel walls.

by Dancis and co-workers [15] for premature infants having the same average birth weight.

It is important to note that the observations and conclusions cited in this report are based on a population of premature infants whose birth weights were 1500 gm. or less and who survived for at least 48 hours (at which time they were admitted to this study). The characteristics of these infants, such as birth weight, gestational age, sex, race, etc., are presented in various Tables and Figures. The results of this study can be used as a basis for judging the probable risk involved in administering oxygen to infants comprising any other population of premature infants having generally similar characteristics. The data are not necessarily comparable, however, with those from other studies, since the latter often include infants having different characteristics, e. g., birth weights of more than 1500 gm.

The results also afford a basis for estimating the probable risk in administering any given amount of oxygen to a particular infant. The multiplicity of birth, birth weight, and, to a lesser extent, gestational age of the infant have, of course, to be taken into account, since each of these factors has been shown to influence the likelihood that an infant will develop RLF. To illustrate, the data cited in Figures 4 and 5 show that greater caution must be exercised by the pediatrician in administering oxygen, if any oxygen at all is required, to an infant of multiple birth than to an infant of single birth. The figures show, too, that, generally speaking, for an infant of multiple birth whose gestational age is less than 33 weeks the risk of exposure to oxygen is probably greater than for an infant born more nearly at term (Fig. 8).

Summary

The chief purposes of the Cooperative Study of Retrolental Fibroplasia (RLF) were to determine, by means of a controlled clinical trial, whether the incidence of RLF was positively associated with the length of exposure of the premature infant to oxygen and whether restricting oxygen to that amount deemed necessary to prevent anoxia would influence the survival rate.

The study was designed so that two groups of premature infants received widely different amounts of oxygen while all other factors associated with the care of the infants were kept as similar as possible.

There were 786 premature infants in the study. These infants represent all those who were born in or brought to the 18 cooperative hospital nurseries during the study year, who weighed 1500 gm. or less at birth, and who survived 48 hours, at which time they were admitted to the study. Of these infants, 586 were followed for a minimum of two and one-half months and represent the study population so far as RLF is concerned.

One-third of the infants in each hospital who were admitted to the study during the first three months were assigned at random to a group which was to be given oxygen for 28 days (routine-oxygen group), and two-thirds were assigned to a group which was to be given oxygen only at the discretion of the pediatrician, on the basis of clinical need (curtailed-oxygen group). The assignments were made in matched sets of three infants. For the remaining nine months of the study all of the infants were assigned to the latter group. There were 53 infants in the routine-oxygen group and 533 infants in the curtailed-oxygen group who were followed sufficiently long to permit a diagnosis. Eye examinations were made at frequent intervals, in most instances until the infant was 6 months of age.

The mortality rates in the routine- and curtailed-oxygen groups did not differ significantly, either during the first three months of the study or throughout the entire year.

The incidence of active stages of RLF in the routine-oxygen group was approximately twice that in the curtailed-oxygen group ($P<0.01$). *The incidence of cicatrical grades* of the disease in the routine-oxygen group was approximately three and a half times that in the curtailed-oxygen group ($P<<0.01$).

The incidence of both active and cicatricial forms of RLF was less for infants of *single birth* than for those of *multiple birth*. The incidence of the cicatricial form of the disease for infants of single birth was only approximately one-third of that for infants of multiple birth ($P<0.01$), despite the fact that infants of single birth received oxygen for two days more, on the average, and had an average gestational age two weeks less than the infants of multiple birth.

The incidence of cicatricial grades of RLF in infants of both single and multiple birth who were in the routine-oxygen group, and whose *health status* therefore can be considered representative of all the infants in the study (classified as the "well" infant group), was approximately twice that for infants who received similarly long stays in oxygen given on the basis of clinical need (classified as the "sick" infant group).

The incidence of both active and cicatricial RLF increased rapidly with increased *duration of exposure to oxygen* for infants of both single and multiple birth. The incidence in infants of single birth increased most rapidly for exposures to oxygen of only *one or two* days. For infants of multiple birth the incidence increased up to periods of exposure to oxygen of about two weeks.

The incidence of cicatricial RLF for the most part was not dependent upon the *concentration of oxygen* administered. Thus, the incidence was not appreciably greater for those infants of single birth who received an average concentration of oxygen of about 33% than for those infants who received an average concentration of oxygen of approximately 50% for similar periods of time. The incidence of cicatricial RLF for infants of multiple birth who received oxygen for relatively short periods of time (two days) likewise was not appreciably influenced by concentration. However, with longer exposures (12 days) it did increase with the average concentration of oxygen administered.

The incidence of cicatricial RLF was not appreciably dependent upon *gestational age* for infants of single birth. The effect of gestational age on the incidence of the disease for infants of multiple birth could not be determined because of variations in duration of exposure to, and concentration of, oxygen, as well as because of differences in birth weights of the infants.

The incidence of cicatricial RLF was inversely related to *birth weight* for all infants.

The incidence of cicatricial RLF was not apparently influenced by *race, sex, type of incubator used, or any of a number of other miscellaneous factors* associated with either the infant or the mother.

The incidence of cicatricial RLF in the group of infants who received no added oxygen after entry into the study (age 48 hours) was less than 1% (1/112). The one infant who developed the disease (Grade A,A) was known to have received 11 hours of oxygen prior to admission to the study.

Exposure to added oxygen (prior to admission to the study) for less than a total of 48 hours was accompanied by some risk of cicatricial RLF (one infant of

52). However, the disease did not progress to the cicatricial grades as frequently as for longer exposures. Moreover, the incidence of active RLF is also reduced (ca. 10%).

The severity of cicatricial RLF was not dependent upon the duration in or concentration of oxygen, gestational age, birth weight, race, sex, or geographical location of the hospital nursery for infants of either single or multiple birth.

Spontaneous regression of RLF from active Stages 1 and 2 occurred in approximately 90% of the cases of infants of single birth. Regression was much less frequent from active Stages 3, 4, and 5 for infants of single birth and a relatively rare occurrence in infants of multiple birth from Stage 2 or greater.

The age of the infant at onset of RLF was not dependent upon the *gestational age or birth weight,* but it increased with the length of stay in oxygen, particularly for longer periods (several weeks or more). Some infants developed the disease while still in oxygen.

The *average weight gain* of infants did not differ widely between groups of infants who did and did not develop cicatricial RLF.

Other factors such as number of *transfusions, and infusions, frequency of medication, dietary adjuncts, complications of pregnancy, and age of mother* did not appear to affect the incidence of the cicatricial form of the disease.

Conclusions

Of the factors considered, this controlled study shows conclusively that the length of time the premature infant is kept in an oxygen-enriched environment is the important factor in the production of RLF.

Limiting the duration in oxygen to that deemed necessary to meet frank clinical emergency was shown to be without effect on the survival rate of the premature infant.

For all practical purposes there is no concentration of oxygen in excess of that in air that is not associated with risk of developing RLF.

Infants of multiple birth appear to be significantly more susceptible to RLF than infants of single birth.

Rate of withdrawal from oxygen does not appear to play a primary role in the pathogenesis of RLF.

Recommendations

The length of time a premature infant, particularly an infant of multiple birth, is kept in an environment containing oxygen in concentrations in excess of that of air should be kept to an absolute minimum, consistent with the clinical indications of anoxia. When oxygen therapy is clearly required, it should be prescribed on an hourly basis and the concentration should be as low as possible.

Dr. Elek Ludvigh, of the Kresge Eye Institute, Detroit, acted as general consultant throughout the study. He assisted greatly, particularly in formulating the design of the study and in analyzing the results.

The late Dr. Jonas S. Friedenwald, of the Wilmer Institute, Baltimore, served as the chairman of the initial ad hoc committee sponsored by the United States Public Health Service, which was instrumental in organizing the study.

Mrs. Mary L. New, School of Public Health, University of Michigan, Ann Arbor, acted as junior statistician in supervising the transcription and tabulation of the data and in making many of the numerous computations used in preparing this report.

Dr. William C. Owens, of the Walter Reed Army Medical Center, Washington, D. C.,

visited several of the hospital nurseries, where, in the interests of minimizing subjective variations in diagnostic classification, he reviewed the ophthalmic findings with the several ophthalmologists.

Dr. Windsor Davies, of the Kresge Eye Institute, Detroit, prepared histologic sections of eyes of premature infants of single and multiple birth and studied them with respect to determining possible differences in the developmental state of the retina.

Dr. Leona Zacharias, of the Massachusetts Eye and Ear Infirmary, Boston, carefully reviewed the manuscript and made many constructive criticisms and suggestions.

Miss Nancy Blair, of the Kresge Eye Institute, Detroit, assisted in the processing and tabulation of the data and in the preparation of this report.

REFERENCES

1. Kinsey, V. E., and Zacharias, L.: Retrolental Fibroplasia: Incidence in Different Localities in Recent Years and a Correlation of the Incidence with Treatment Given the Infants, J. A. M. A. 139:572, 1949.

2. Campbell, K.: Intensive Oxygen Therapy as a Possible Cause of Retrolental Fibroplasia: A Clinical Approach, M. J. Australia 2:48, 1951.

3. Crosse, V. M., and Evans, P. J.: Prevention of Retrolental Fibroplasia, A. M. A. Arch. Ophth. 48:83, 1952.

4. Ryan, H.: Retrolental Fibroplasia: A Clinicopathologic Study, Am. J. Ophth. 35:329, 1952.

5. Jefferson, E.: Retrolental Fibroplasia, Arch. Dis. Childhood 27:329, 1952.

6. Patz, A.; Hoeck, L. E., and De La Cruz, E.: Studies on the Effect of High Oxygen Administration in Retrolental Fibroplasia: I. Nursery Observations, Am. J. Ophth. 35:1248, 1952.

7. Gyllensten, L. J., and Hellström, B. E.: Retrolental Fibroplasia—Animal Experiments: The Effect of Intermittingly Administered Oxygen on the Postnatal Development of the Eyes of Fullterm Mice; A Preliminary Report, Acta pediat. 41:577, 1952.

8. Kinsey, V. E., and Hemphill, F. M. Etiology of Retrolental Fibroplasia: Preliminary Report of a Cooperative Study of Retrolental Fibroplasia, Am. J. Ophth. 40:166 (Aug.); Tr. Am. Acad. Ophth. 59:15, 1955.

9. Zacharias, L.: Retrolental Fibroplasia: A Survey, Am. J. Ophth. 35:1426, 1952.

10. Reese, A. B.; King, M., and Owens, W. C.: A Classification of Retrolental Fibroplasia, Am. J. Ophth. 36:1333, 1953.

11. Silverman, W. A.; Blodi, F. C.; Locke, J. C.; Day, R. L., and Reese, A. B.: Incidence of Retrolental Fibroplasia in a New York Nursery, A. M. A. Arch. Ophth. 48:698, 1952.

12. Guy, L. P.; Lanman, J. T., and Dancis, J.: The Possibility of Total Elimination of Retrolental Fibroplasia by Oxygen Restriction, Pediatrics 17:247, 1956.

13. Patz, A.: Oxygen Studies in Retrolental Fibroplasia: IV. Clinical and Experimental Observations, Am. J. Ophth. 38:291, 1954.

14. Michaelson, I. C.: Retinal Circulation in Man and Animals, Springfield, Ill., Charles C Thomas, Publisher, 1954.

15. Dancis, J.; O'Connell, J. R., and Holt, L. E., Jr.: A Grid for Recording the Weight of Premature Infants, J. Pediat. 33:570, 1948.

Appendix

All items MUST be answered or checked. When information cannot be supplied, write in: O. K. (don't know)

COOPERATIVE STUDY OF ENVIRONMENTAL OXYGEN AND RETROLENTAL FIBROPLASIA

Record to be filled out on admission to study

R.L.P. Study Record N? 671

NAME OF PARENTS

NAME OF HOSPITAL — HOSPITAL RECORD NO.

ADDRESS — NUMBER — STREET — R.F.D.

PLACE OF BIRTH IF BORN OUTSIDE THIS HOSPITAL — NAME OF HOSPITAL AND CITY

CITY — ZONE — STATE

TELEPHONE NUMBER WHERE PARENT MAY BE REACHED

☐ HERE ☐ ELSEWHERE — SPECIFY

INFORMATION CONCERNING PREMATURE INFANT

☐ A.M. ☐ P.M. 195__ Birth date — ☐ MALE ☐ FEMALE

MONTH — DAY — HOUR

Birth weight — ☐ ACTUAL ☐ ESTIMATE

DAY — HOUR ☐ A.M. ☐ P.M. Date of weighing

☐ SINGLE
☐ TWIN
☐ TRIPLET
☐ QUADRUPLET
— SINGLE OR MULTIPLE BIRTH

☐☐☐ MALE
☐☐☐ FEMALE
— SEX OF OTHER TWIN, TRIPLETS OR QUADRUPLETS

☐ NONE
SPECIFY — Resuscitation method used

☐ NONE
SPECIFY — Type of abnormality

INFORMATION CONCERNING PARENTS

YEARS — Age of mother

WHITE ☐ NEGRO ☐ OTHER ☐ SPECIFY — Race of Mother

☐ ☐ ☐ SPECIFY — Race of Father

SPECIFY — Nationality of Mother

SPECIFY — Nationality of Father

MONTH — DAY — 195__ Date of last menstrual period

SPECIFY — Type of delivery

SPECIFY — Cause of prematurity

SPECIFY — Kind of anesthetic

HOURS — Duration of anesthetic

SPECIFY — Kind of analgesic

☐ None
☐ Infection
☐ Bleeding
☐ Chronic disease
☐ Toxemia
SPECIFY — ☐ Other

COMPLICATIONS OF PREGNANCY

(Please indicate whether 1st, 2nd or 3rd trimester by placing number in appropriate box.)

At end of week mail ORIGINAL of this sheet to Coordinating Center (Knapp Eye Institute) and retain SECOND COPY for hospital. PLEASE DO NOT FOLD IN MAILING

Figure 1A

All items MUST be answered or checked. When information cannot be supplied, write in: D. K. (don't know)

WEEKLY RECORD
(Curtailed Oxygen Group)

R.L.F. Study Record No. 671

Weight _____ Date _____ (MONTH – DAY) 195__ Baby _____ (NAME)

IF, FOR ANY REASON, BABY IS PLACED IN OXYGEN, PLEASE INDICATE BY FILLING IN THIS SECTION

DATE	HOUR PLACED IN OXYGEN	HOUR REMOVED FROM OXYGEN	FLOW RATE L/MIN.	CONC. OXYGEN	REASON FOR ADMINISTRATION OF OXYGEN
	☐ A.M. ☐ P.M.	☐ A.M. ☐ P.M.		%	
	☐ A.M. ☐ P.M.	☐ A.M. ☐ P.M.		%	
	☐ A.M. ☐ P.M.	☐ A.M. ☐ P.M.		%	

DAILY RECORD OF OXYGEN CONCENTRATION AND HUMIDITY: ROUTINE OXYGEN GROUP

INSERT DAY OF MONTH ==>	SUNDAY (DATE)		MONDAY (DATE)		TUESDAY (DATE)		WEDNESDAY (DATE)		THURSDAY (DATE)		FRIDAY (DATE)		SATURDAY (DATE)	
	OXYGEN CONCEN.	HUMIDITY	OXYGEN CONCEN.	HUMIDITY	OXYGEN CONCEN.	HUMIDITY	OXYGEN CONCEN.	HUMIDITY	OXYGEN CONCEN.	HUMIDITY	OXYGEN CONCEN.	HUMIDITY	OXYGEN CONCEN.	HUMIDITY
MORNING	%		%		%		%		%		%		%	
NOON	%		%		%		%		%		%		%	
NIGHT	%		%		%		%		%		%		%	

TYPE OF INCUBATOR

☐ Isolette
☐ Gordon Armstrong
_____ Other (SPECIFY)

METHOD OF FEEDING

☐ Gavage
☐ Nipple
Polyethylene

TIME FOR FEEDING (Average for week)

☐ Under 5 mins.
☐ 5-10 mins.
☐ Over 10 mins.

_____ Number of feedings per day (average for week)

INFUSIONS

☐ NONE

How Given	Dosage		No. of Infusions	Dates
☐ I.V. ☐ S.C.	_____ (CC)	Glucose	_____	_____
☐ I.V. ☐ S.C.	_____ (CC)	Saline	_____	_____
☐ I.V. ☐ S.C.	_____ (CC)	_____ (SPECIFY)	_____	_____

☐ NONE **TRANSFUSIONS**

DOSAGE	NO. TRANSFUSIONS	DATES
_____ (CC/KG)	_____	_____

(TIME SERVICE INC.—N.Y.—37)

EYE EXAMINATION

DATE	DEGREE OF RETROLENTAL FIBROPLASIA OS	OD

NAME OF OPHTHALMOLOGIST

Remarks _____

☐ NONE ***CHEMOTHERAPY & ANTIBIOTICS**

_____ (KIND) _____ (DOSAGE) from _____ (DATE) to _____ (DATE)
_____ (KIND) _____ (DOSAGE) from _____ (DATE) to _____ (DATE)

☐ NONE ***OTHER MEDICATION**

_____ (KIND) _____ (DOSAGE) from _____ (DATE) to _____ (DATE)
_____ (KIND) _____ (DOSAGE) from _____ (DATE) to _____ (DATE)
_____ (KIND) _____ (DOSAGE) from _____ (DATE) to _____ (DATE)

*Reason for medication & remarks on general condition of infant:

DIETARY ADJUNCTS (If other than specified in protocol)

Type of Food _____
Vitamins _____ (KIND) _____ (DOSAGE) Iron _____ (KIND) _____ (DOSAGE)

IF DECEASED Date _____ (MONTH) _____ (DAY) 195__

Autopsy ☐ YES ☐ NO

Disposition of Eyes _____ (WHERE SENT)

At end of week mail ORIGINAL of this sheet to Coordination Center (Kresge Eye Institute) and retain SECOND COPY for hospital.
PLEASE DO NOT FOLD IN MAILING

Figure 2A

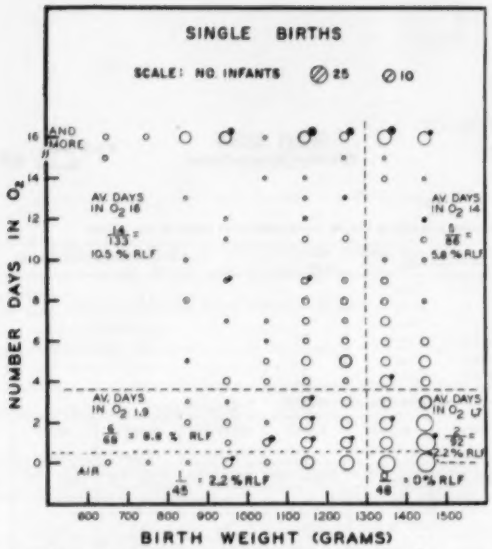

SINGLE BIRTHS
SCALE: NO. INFANTS 25 10
NUMBER DAYS IN O2
16 AND MORE
AV. DAYS IN O2 16
14/133 = 10.5 % RLF
AV. DAYS IN O2 14
5/86 = 5.8 % RLF
AV. DAYS IN O2 1.9
6/68 = 8.8 % RLF
AV. DAYS IN O2 1.7
2/92 = 2.2% RLF
A/R
1/45 = 2.2 % RLF
0/48 = 0% RLF
600 700 800 900 1000 1100 1200 1300 1400 1500
BIRTH WEIGHT (GRAMS)

Fig. 3A.—Infants of single birth arranged according to birth weight and the number of days in oxygen. Area of the circles represents number of infants; filled circles represent infants who developed cicatricial RLF.

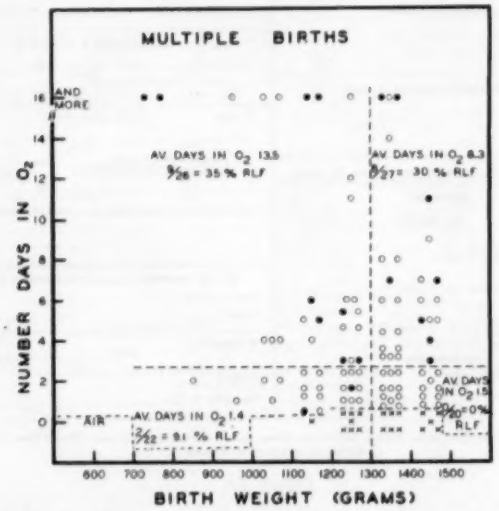

MULTIPLE BIRTHS
NUMBER DAYS IN O2
16 AND MORE
AV. DAYS IN O2 13.5
9/26 = 35 % RLF
AV. DAYS IN O2 8.3
8/27 = 30 % RLF
AV. DAYS IN O2 1.5
20/20 = 0% RLF
A/R
AV. DAYS IN O2 1.4
2/22 = 9.1 % RLF
600 700 800 900 1000 1100 1200 1300 1400 1500
BIRTH WEIGHT (GRAMS)

Fig. 4A.—Infants of multiple birth arranged according to birth weight and number of days in oxygen. Each circle represents one infant. Open circles refer to infants whose eyes did not develop cicatricial RLF; filled circles refer to infants whose eyes developed cicatricial RLF. Crosses refer to infants who received no added oxygen.

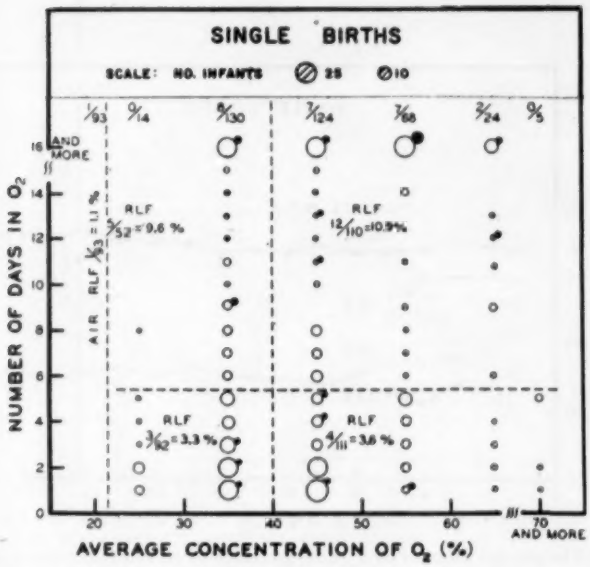


Fig. 5A.—Infants of single birth arranged according to average concentration of oxygen received and number of days in oxygen. Area of the circles corresponds to number of infants; filled circles represent infants who developed cicatricial RLF.

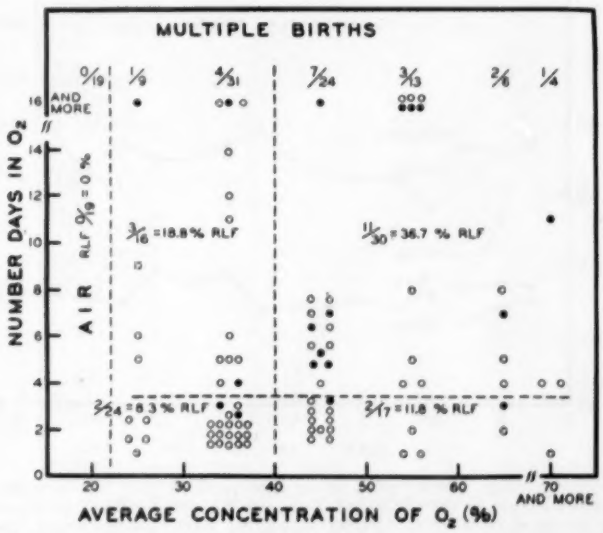


Fig. 6A.—Infants of multiple birth arranged according to average concentration of oxygen received and number of days in oxygen. Each circle represents one infant. Open circles refer to infants whose eyes did not develop cicatricial RLF; filled circles refer to infants who developed cicatricial RLF.

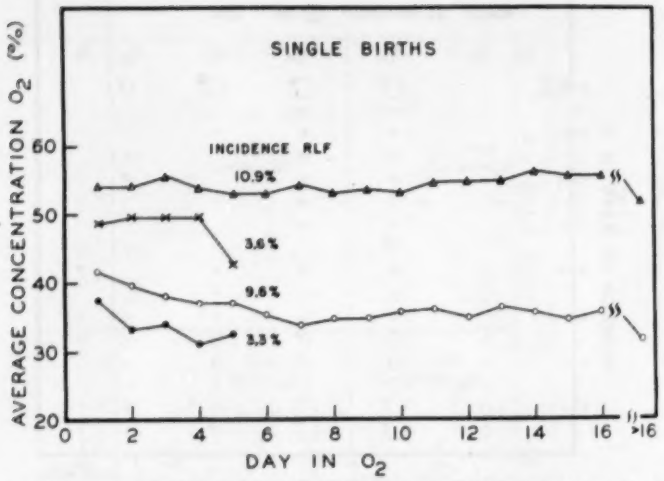


Fig. 7A.—The average concentration of oxygen received each day by each of the four groups of infants shown in the quadrants of Figure 5A and incidence of cicatricial RLF in each of these groups of infants. The Figure shows that the average concentration of oxygen did not vary appreciably from day to day.

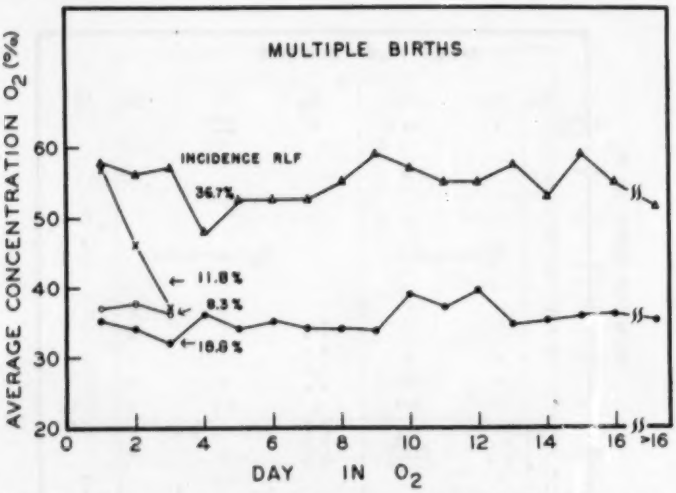


Fig. 8A.—The average concentration of oxygen received each day by each of the four groups of infants shown in the quadrants of Figure 6A and incidence of cicatricial RLF in each of these groups of infants. The Figure shows that except for one group (line of crosses) the average concentration of oxygen did not vary appreciably from day to day.

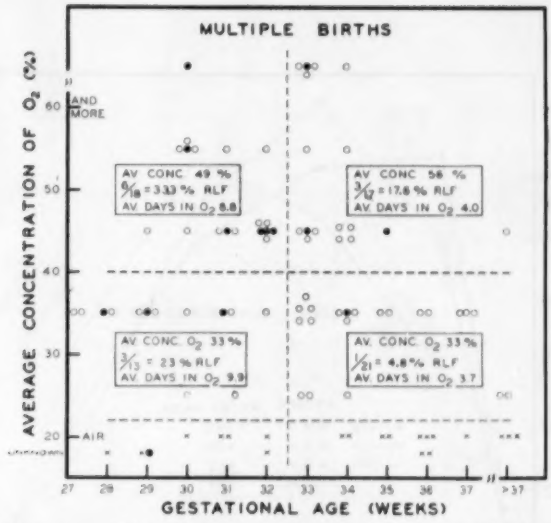

MULTIPLE BIRTHS
AVERAGE CONCENTRATION OF O_2 (%)
60 AND MORE
50
40
30
20 AIR
AV. CONC. 49 %
9/18 = 33.3 % RLF
AV. DAYS IN O_2 8.8
AV. CONC. 58 %
3/17 = 17.6 % RLF
AV. DAYS IN O_2 4.0
AV. CONC. O_2 33 %
3/13 = 23 % RLF
AV. DAYS IN O_2 9.9
AV. CONC. O_2 33 %
1/21 = 4.8 % RLF
AV. DAYS IN O_2 3.7
27 28 29 30 31 32 33 34 35 36 37 >37
GESTATIONAL AGE (WEEKS)

Fig. 9A.—Infants of multiple birth arranged according to gestational age and average concentration of oxygen received. Each circle represents one infant. The open circles refer to infants whose eyes did not develop cicatricial RLF; filled circles refer to infants whose eyes developed cicatricial RLF. The crosses refer to infants who either received no added oxygen or whose concentration of oxygen was unknown.

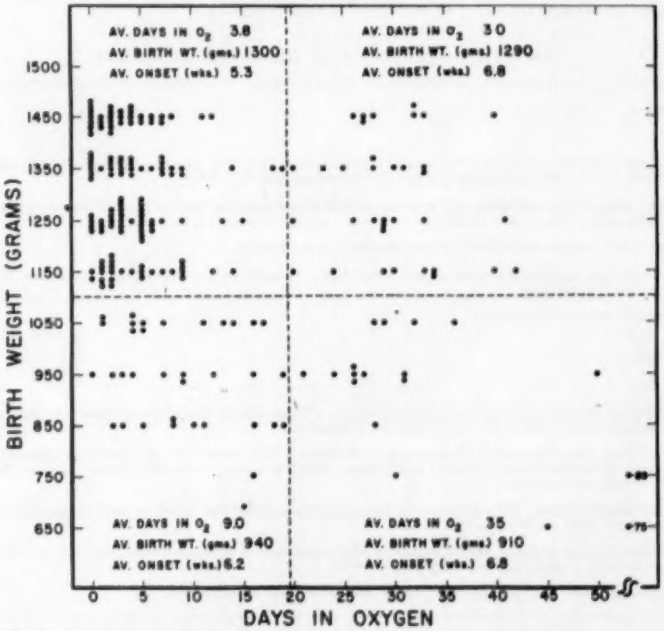

AV. DAYS IN O_2 3.8
AV. BIRTH WT. (gms.) 1300
AV. ONSET (wks.) 5.3
AV. DAYS IN O_2 3.0
AV. BIRTH WT. (gms.) 1290
AV. ONSET (wks.) 6.8
AV. DAYS IN O_2 9.0
AV. BIRTH WT. (gms.) 940
AV. ONSET (wks.) 6.2
AV. DAYS IN O_2 35
AV. BIRTH WT. (gms.) 910
AV. ONSET (wks.) 6.8
BIRTH WEIGHT (GRAMS)
1500 1450 1350 1250 1150 1050 950 850 750 650
0 5 10 15 20 25 30 35 40 45 50
DAYS IN OXYGEN

Fig. 10A.—Infants who developed active RLF arranged according to birth weight and number of days in oxygen. Filled circles represent infants of either single or of multiple birth.

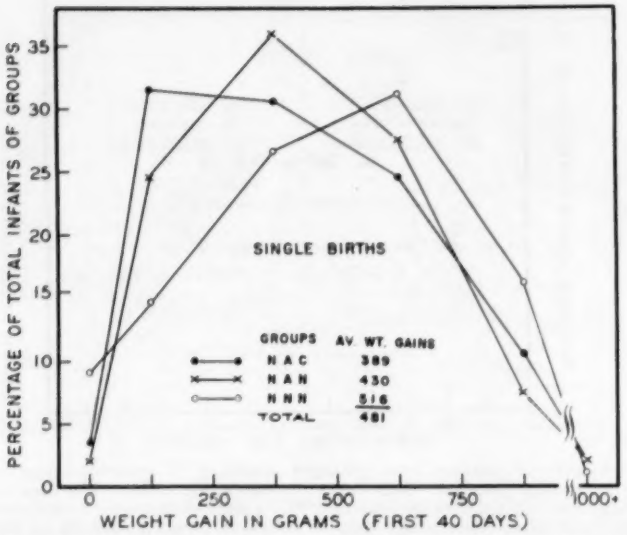


Fig. 11A.—Infants arranged according to weight gained during the first 40 days of life depending upon whether their eyes developed cicatricial RLF (NAC), developed active RLF and regressed to normal (NAN), or remained normal (NNN).

TABLE 1A.—*Classification of Retrolental Fibroplasia*

Active Phase:

Stage

1. Dilatation and tortuosity of retinal vessels; hemorrhages may or may not be present; early neovascularization, especially in the extreme periphery of the visible fundus, may be present
2. Stage 1 plus neovascularization and some peripheral retinal clouding; hemorrhages are usually present; vitreous clouding may or may not be present
3. Stage 2 plus retinal detachment in the periphery of the fundus
4. Hemispherical or circumferential retinal detachment; elevation of the retina over a large area, but still with some retina in position
5. Complete retinal detachment

Cicatricial Phase:

Grade

A Small mass of opaque tissue in periphery of fundus without visible retinal detachment; the fundus may have a pale appearance; the blood vessels may be attenuated

B Larger mass of opaque tissue in periphery of the fundus with some localized retinal detachment; the disc is distorted by traction toward the side of the tissue, which is usually temporally located; cases ending in Grade A or B have useful vision

C Larger mass of opaque tissue in periphery incorporating a retinal fold which extends to the disc; visual acuity varies from 5/200 to 20/50

D Retrolental tissue covering part of pupillary area; small area of attached retina may still be visible or only a rod reflex over a sector of the fundus may be seen

E Retrolental tissue covering entire pupillary area; no fundus reflex present

TABLE 2A.—*Characteristics of Infants Whose Eyes were Examined for a Minimum of Two and One-Half Months (Study Population)*

Hospital	Total Infants		Race				Weight Group, Gm.			Place of Birth			Protocol Group	
			White		Nonwhite									
			M	F	M	F	1,000 & Less	1,001-1,250	1,251-1,500	This Hosp.	Another Hosp.	Elsewhere	Routine O_2	Curtailed O_2
Abington Memorial Hospital	Sing.	3	--	1	--	2	--	1	2	3	--	--	--	3
	Mult.	--	--	--	--	--	--	--	--	--	--	--	--	--
Baltimore City Hospital	Sing.	22	1	2	6	13	1	4	17	22	--	--	1	21
	Mult.	3	--	--	1	2	--	--	3	3	--	--	--	3
Boston Lying-In Hospital	Sing.	9	3	5	--	1	--	2	7	9	--	--	1	8
	Mult.	2	1	1	--	--	--	1	1	2	--	--	--	2
Cincinnati General Hospital	Sing.	32	6	5	10	11	3	14	15	25	3	4	3	29
	Mult.	3	1	--	2	--	2	--	1	2	1	--	--	3
Children's (and affil.) Hospital (Columbus)	Sing.	27	11	9	1	6	2	4	21	4	22	1	--	27
	Mult.	4	1	3	--	--	1	1	2	--	4	--	--	4
Babies' and Children's Hospital (Univ. Hosp. of Cleveland)	Sing.	25	1	4	10	10	3	8	14	23	--	2	4	21
	Mult.	10	--	--	4	6	--	1	9	10	--	--	--	10
Cooper Hospital	Sing.	4	1	1	--	2	1	--	3	4	--	--	--	4
	Mult.	3	2	1	--	--	--	--	3	3	--	--	--	3
Chicago Lying-In Hospital	Sing.	12	4	4	--	4	2	7	3	12	--	--	2	10
	Mult.	2	--	1	--	1	--	2	--	2	--	--	--	2
Michael Reese Hospital	Sing.	55	11	16	11	17	8	17	30	11	26	18	7	48
	Mult.	11	4	1	5	1	--	5	6	2	5	4	1	10
Charity Hospital of Louisiana	Sing.	127	11	12	30	34	16	43	68	66	30	31	16	111
	Mult.	36	5	2	11	18	2	11	23	18	13	5	3	33

TABLE 2A.—*Characteristics of Infants Whose Eyes were Examined for a Minimum of Two and One-Half Months (Study Population)*—Continued

Hospital	Total Infants		Race				Weight Group, Gm.			Place of Birth			Protocol Group	
			White		Nonwhite									
			M	F	M	F	1,000 & Less	1,001-1,250	1,251-1,500	This Hosp.	Another Hosp.	Elsewhere	Routine O_2	Curtailed O_2
Babies' Hospital (New York)	Sing.	58	22	23	3	10	9	21	28	3	54	1	6	52
	Mult.	8	1	4	--	3	--	3	5	--	8	--	--	8
Bellevue Hospital	Sing.	21	5	12	1	3	4	9	8	8	11	2	--	21
	Mult.	4	1	3	--	--	--	1	3	1	3	--	1	3
New York Hospital	Sing.	25	7	14	1	3	5	9	11	6	18	1	2	23
	Mult.	12	4	4	2	2	--	4	8	2	10	--	--	12
Hospital of the University of Pennsylvania	Sing.	7	--	2	3	2	--	4	3	4	3	--	1	6
	Mult.	4	--	1	--	3	--	3	1	4	--	--	--	4
Pennsylvania Hospital	Sing.	11	--	2	5	4	--	5	6	11	--	--	1	10
	Mult.	--	--	--	--	--	--	--	--	--	--	--	--	--
Temple University Hospital	Sing.	15	2	2	5	6	1	4	10	12	1	2	2	13
	Mult.	5	--	--	1	4	--	2	3	5	--	--	--	5
Elizabeth Steel Magee Hospital	Sing.	17	7	5	3	2	2	4	11	17	--	--	1	16
	Mult.	5	--	3	2	--	--	2	3	5	--	--	--	5
Walter Reed Army Hospital	Sing.	2	--	1	--	1	1	--	1	2	--	--	--	2
	Mult.	2	--	--	--	2	--	2	--	2	--	--	1	1
Total	Sing.	472	92	120	109	151	58	156	258	242	168	62	47	425
	Mult.	114	20	24	28	42	5	38	71	61	44	9	6	108
Grand total	Sing. and Mult.	586	112	144	137	193	63	194	329	303	212	71	53	533

TABLE 3A.—*Characteristics of Infants Admitted to Study Who Died Prior to Forty Days of Age*

Hospital		Total No. Infants	Race				Weight Group, Gm.			Place of Birth			Protocol Group	
			White		Nonwhite									
			M	F	M	F	1,000 & Less	1,001-1,250	1,251-1,500	This Hosp.	Another Hosp.	Elsewhere	Routine O_2	Curtailed O_2
Abington Memorial Hospital	Sing.	--	--	--	--	--	--	--	--	--	--	--	--	--
	Mult.	--	--	--	--	--	--	--	--	--	--	--	--	--
Baltimore City Hospital	Sing.	12	1	--	7	4	6	4	2	12	--	--	1	11
	Mult.	1	--	--	--	1	1	--	--	1	--	--	--	1
Boston Lying-In Hospital	Sing.	2	1	--	--	1	--	2	--	2	--	--	1	1
	Mult.	2	2	--	--	--	--	2	--	2	--	--	--	2
Cincinnati General Hospital	Sing.	5	1	--	1	3	3	1	1	3	1	1	--	5
	Mult.	1	--	--	1	--	--	1	--	1	--	--	--	1
Children's (and affil.) Hospital (Columbus)	Sing.	12	5	4	1	2	4	3	5	2	8	2	1	11
	Mult.	2	1	1	--	--	--	2	--	--	2	--	--	2
Babies' and Children's Hosp. (Univ. Hosp. of Cleveland)	Sing.	11	1	2	3	5	4	3	4	11	--	--	1	10
	Mult.	--	--	--	--	--	--	--	--	--	--	--	--	--
Cooper Hospital	Sing.	2	1	--	1	--	1	1	--	--	--	2	--	2
	Mult.	--	--	--	--	--	--	--	--	--	--	--	--	--
Chicago Lying-In Hospital	Sing.	3	2	--	1	--	--	2	1	3	--	--	--	3
	Mult.	--	--	--	--	--	--	--	--	--	--	--	--	--
Michael Reese Hospital	Sing.	8	5	1	1	1	4	3	1	1	4	3	1	7
	Mult.	2	--	2	--	--	--	1	1	--	1	1	--	2
Charity Hospital of Louisiana	Sing.	22	6	4	7	5	12	8	2	14	6	2	2	20
	Mult.	6	--	2	1	3	5	1	--	3	2	1	1	5

TABLE 3A.—*Characteristics of Infants Admitted to Study Who Died Prior to Forty Days of Age*—Continued

Hospital	Total No. Infants		Race				Weight Group, Gm.			Place of Birth			Protocol Group	
			White		Nonwhite									
			M	F	M	F	1,000 & Less	1,001-1,250	1,251-1,500	This Hosp.	Another Hosp.	Elsewhere	Routine O_2	Curtailed O_2
Babies' Hospital (New York)	Sing.	44	22	14	6	2	17	13	14	--	43	1	3	41
	Mult.	5	2	3	--	--	--	2	3	--	4	1	--	5
Bellevue Hospital	Sing.	7	5	2	--	--	2	5	--	4	2	1	3	4
	Mult.	2	--	2	--	--	2	--	--	1	1	--	--	2
New York Hospital	Sing.	2	1	1	--	--	1	1	--	1	1	--	--	2
	Mult.	--	--	--	--	--	--	--	--	--	--	--	--	--
Hospital of the University of Pennsylvania	Sing.	2	1	--	--	1	--	2	--	1	--	1	--	2
	Mult.	1	--	--	--	1	--	--	1	1	--	--	1	--
Pennsylvania Hospital	Sing.	4	--	--	2	2	--	3	1	4	--	--	--	4
	Mult.	--	--	--	--	--	--	--	--	--	--	--	--	--
Temple University Hospital	Sing.	--	--	--	--	--	--	--	--	--	--	--	--	--
	Mult.	1	--	--	--	1	--	--	1	1	--	--	--	1
Elizabeth Steel Magee Hospital	Sing.	5	2	1	2	--	2	--	3	5	--	--	--	5
	Mult.	--	--	--	--	--	--	--	--	--	--	--	--	--
Walter Reed Army Hospital	Sing.	1	1	--	--	--	1	--	--	1	--	--	--	1
	Mult.	1	1	--	--	--	1	--	--	1	--	--	--	1
Total	Sing.	142	55	29	32	26	57	51	34	64	65	13	13	129
	Mult.	24	6	10	2	6	9	9	6	11	10	3	2	22
Grand total	Sing. and Mult.	166	61	39	34	32	66	60	40	75	75	16	15	151

TABLE 4A.—*Characteristics of Infants Who Died Between Forty and Seventy-Five Days of Age or Who Could Not Be Followed for a Minimum of Seventy-Five Days*

Birth	No. Infants	Race				Weight Group			Place of Birth			Protocol Group	
		White		Nonwhite		1,000 & Less	1,001-1,250	1,251-1,500	This Hosp.	Another Hosp.	Elsewhere	Routine O_2	Curtailed O_2
		M	F	M	F								
Infants who died between 40 and 75 days of age													
Single births	4	1	0	2	1	0	3	1	3	0	1	0	4
Multiple births	3	0	0	2	1	0	2	1	1	1	1	0	3
Total	7	1	0	4	2	0	5	2	4	1	2	0	7
Infants who could not be followed for a minimum of 75 days													
Single births	25	5	6	10	4	1	8	16	13	10	2	0	25
Multiple births	2	1	1	..	..	0	1	1	1	1	..	0	2
Total	27	6	7	10	4	1	9	17	14	11	2	0	27

TABLE 5A.—*Mortality* According to Oxygen Group, by Hospital*

Hospital	Routine-Oxygen Group	Curtailed-Oxygen Group
	Mortality, %	Mortality, %
Abington Memorial Hospital	0 (0/0)	0 (0/3)
Baltimore City Hospital	50 (1/2)	32 (12/37)
Boston Lying-In Hospital	50 (1/2)	23 (3/13)
Cincinnati General Hospital	0 (0/3)	15 (6/41)
Children's (and affiliated) Hospital, Columbus	100 (1/1)	26 (13/49)
Babies' and Children's Hospital (University Hospitals of Cleveland)	20 (1/5)	24 (10/41)
Cooper Hospital	0 (0/0)	20 (2/10)
Chicago Lying-In Hospital	0 (0/2)	20 (3/15)
Michael Reese Hospital	11 (1/9)	13 (9/69)
Charity Hospital of Louisiana	14 (3/22)	14 (25/178)
Babies' Hospital (New York)	33 (3/9)	42 (46/110)
Bellevue Hospital	75 (3/4)	19 (6/31)
New York Hospital	0 (0/2)	5 (2/40)
Hospital of the University of Pennsylvania	50 (1/2)	17 (2/12)
Pennsylvania Hospital	0 (0/1)	29 (4/14)
Temple University Hospital	0 (0/2)	5 (1/20)
Elizabeth Steel Magee Hospital	0 (0/1)	17 (5/30)
Walter Reed Army Hospital	0 (0/1)	40 (2/5)
All Hospitals	22 (15/68)	21 (151/718)

* All figures refer to mortality after admission to the study, at 2 days of age.

TABLE 6A.—*Incidence of Active and Cicatricial Retrolental Fibroplasia in Curtailed-Oxygen Group According to Trimester of the Study*

Trimester of Study	Incidence of Active RLF (All Stages), %	
	Single Births	Multiple Births
1	29 (24/82)	45 (10/22)
2	32 (38/120)	48 (11/23)
3	26 (26/99)	31 (10/32)
4	36 (45/124)	45 (14/31)
1-4 (12 mo.)	31 (133/425)	42 (45/108)
	Cicatricial RLF (All Grades)	
1	4.9 (4/82)	18 (4/22)
2	4.2 (5/120)	13 (3/23)
3	3.0 (3/99)	9.4 (3/32)
4	6.4 (8/124)	16 (5/31)
1-4 (12 mo.)	4.7 (20/425)	14 (15/108)

TABLE 7A.—*Active Stages and Cicatricial Grades of Retrolental Fibroplasia in Infants of Single and Multiple Births, by Hospital*

Hospital	Single Births, No. of Infants			Multiple Births, No. of Infants		
	Active Stages	Cicatricial Grades	Total of Single Birth	Active Stages	Cicatricial Grades	Total of Multiple Birth
Abington Memorial Hospital	0	0	3	0	0	0
Baltimore City Hospital	6	3	22	0	0	3
Boston Lying-In Hospital	6	0	9	1	0	2
Cincinnati General Hospital	7	3	32	1	1	3
Children's (and affiliated) Hospital (Columbus)	3	0	27	3	2	4
Babies' and Children's Hospital (University Hospitals of Cleveland)	4	2	25	3	0	10
Cooper Hospital	1	0	4	0	0	3
Chicago Lying-In Hospital	7	3	12	0	0	2
Michael Reese Hospital	14	2	55	1	0	11
Charity Hospital of Louisiana	69	4	127	21	8	36
Babies' Hospital (New York)	9	2	58	0	0	8
Bellevue Hospital	0	0	21	1	1	4
New York Hospital	21	6	25	10	3	12
Hospital of the University of Pennsylvania	0	0	7	2	1	4
Pennsylvania Hospital	2	0	11	0	0	0
Temple University Hospital	5	1	15	3	1	5
Elizabeth Steel Magee Hospital	12	2	17	3	1	5
Walter Reed Army Hospital	0	0	2	1	1	2
Total	166	28	472	50	19	114

TABLE 8A.—*Incidence of Active Retrolental Fibroplasia According To Stay in Oxygen Before* Admission to the Study*

Hours in Oxygen	Incidence of Active RLF,† %
0	(Unknown no.)
1-12	12 (2/17)
13-24	10 (1/10)
25-48	12 (3/25)

* Infants received no added oxygen after admission at age 48 hours.
† One infant developed active Stages 3 and 4 and finally cicatricial Grade A. All others developed active RLF Stage 2 or less in poorer eye.

Collagen Diseases

General Manifestations and Symptoms of Collagen Diseases

FRANK H. CONSTANTINE, M.D.
New York

A noteworthy group of maladies of unknown origin which primarily involve connective tissue has been termed collagen diseases. These include the conditions known as periarteritis nodosa or polyarteritis, dermatomyositis, scleroderma, and disseminated lupus erythematosus. There are, indeed, several other diseases, such as rheumatoid arthritis, rheumatic fever, and serum sickness, which exhibit structural changes in collagen but of a somewhat limited scope. These can be distinguished from the group of diffuse alterations of collagen under present consideration.

For the purpose of orientation I will very briefly outline the general pathology of these afflictions.

Periarteritis nodosa: widespread focal necrosis and obliteration of the walls of the medium- and small-sized arteries.

Dermatomyositis: chronic nonsuppurative inflammation of the skin and muscles.

Scleroderma: thickening of collagen fibers throughout the body, especially in the skin.

Disseminated lupus erythematosus: degenerative changes in connective tissue of the vascular system, also in serous and synovial membranes.

Similar but nonspecific general manifestations are present in all these diseases. The abnormalities are widespread throughout the body. Fever, malaise, skin, joint, and other symptoms are common to each malady. Because of this, early in the course of the disease the exact diagnosis is often obscure. This group is also characterized by a gradual mode of onset and by remissions and exacerbations of symptoms.

This portion of the symposium will discuss those systems, except the ocular, commonly disturbed by each of these afflictions, that is, the dermatologic, articular, muscular, cardiovascular, renal, gastrointestinal, pulmonary, and nervous systems. The signs and symptoms arising from collagen diseases of these parts will be described and compared.

Dermatologic Manifestations

All of these diseases produce alterations in the skin. In polyarteritis or periarteritis nodosa the least common skin lesion is the subcutaneous nodule to

Received for publication March 22, 1956.
Read in the Symposium on Collagen Diseases at the Fifth Pan-American Congress of Ophthalmology, Santiago, Chile, Jan. 9, 1956.

which the disease owes its name. Palpable aneurysmal dilitations of weakened arteries are extremely rare. Much commoner are multiple purpuric and hemorrhagic extravasations which coalesce and produce large necrotic ulcers.

The most characteristic dermatologic lesion of dermatomyositis is induration and edema of the skin overlying the diseased muscle. This swelling commonly produces a tense edema of the eye lids, with or without a peculiar blue-brown pigmentation. This latter condition has been termed "heliotrope lids." Any other area of skin may exhibit a variety of cutaneous lesions, none of which are specific.

The earliest skin manifestations of scleroderma occur in the face and upper extremities. Classically there is firm nonpitting induration of the skin of the cheeks or of the exposed "V" area of the chest. A smooth waxy or ivory appearance of the affected skin results from a loss of skin wrinkles and pits. Raynaud's syndrome or cold cyanotic extremities may precede dermatologic signs by five years or more.

In lupus erythematosus skin lesions are frequent, and they are so characteristic that they often point the way to the correct diagnosis. In 50% of patients an erythematous rash appears on the more exposed portions of the body, especially on the bridge of the nose, and extends across the malar eminences.

Joint and Muscle Manifestations

The arthralgia of periarteritis is low-grade and fleeting and only casually resembles rheumatoid arthritis. Muscle tenderness, however, may be severe enough to suggest trichinosis or dermatomyositis.

In dermatomyositis the joint symptoms closely resemble those found in periarteritis nodosa. The most prominent features of this disease are attributable to diffuse myositis. Decreased strength of shoulder girdle and upper arm is frequent. These unfortunate patients may be unable to wash their face or comb their hair. The involved muscles are painful and exquisitely tender to pressure. Dyspnea ensues if the diaphragm is damaged.

Those patients with scleroderma typically display trophic changes in the phalanges, with ulceration of skin and marked limitation of hand motion. Bits of calcium may be extruded from the finger tips. The typical end-result is a "claw hand." The muscular system is not primarily affected.

Nearly all patients with disseminated lupus experience polyarthritis closely resembling that seen in rheumatoid arthritis or rheumatic fever. Characteristically the arthralgia is migratory and transitory. Rarely is there any redness, and there is never any deformity. The muscles are not involved in lupus.

Cardiovascular-Renal Manifestations

Seventy-five per cent of patients with periarteritis develop necrosis of renal vessels which leads to progressive hypertension. This is signified by the appearance of albumin, casts, hematuria, dysuria, and pain. These patients also commonly exhibit signs and symptoms of congestive heart failure.

Dermatomyositis is rarely complicated by pathological changes in the heart or kidneys except terminally.

In patients suffering from scleroderma, late in the disease overgrowth of fibrous tissue in the heart results in cardiac failure and pulmonary congestion. This disease does not involve the renal system.

About 30% of those with disseminated lupus have endocardial and myocardial involvement, evidenced by a systolic apical murmur, tachycardia, and cardiac enlargement. Congestive heart failure and hypertension practically never occur. Hematuria and albuminuria are almost universal. These urinary findings superimposed on symptoms of rheumatoid arthritis facilitate the correct diagnosis.

Gastrointestinal Manifestations

Periarteritis can impair the blood supply of any abdominal viscus. That this is so enables it to become the perfect mimic of any of the multitudinous inflammatory diseases which attack the organs in the abdominal cavity. Pain in this region is prominent at one time or another in 50% of patients. Surgical exploration may be indicated. Indeed, the pathologist's examination of the offending gall bladder or appendix may give the first clue to the diagnosis.

Dermatomyositis rarely involves other than striated muscle.

Scleroderma, on the other hand, exhibits very prominent gastrointestinal symptoms. The most characteristic are those involving the esophagus. Dysphagia is the important symptom and may be great enough to mask the rest of the clinical picture.

Some patients with disseminated lupus experience abdominal pain and diarrhea which is associated with fresh vascular lesions in the intestinal wall.

Pulmonary Manifestations

Although those who originally described periarteritis nodosa felt that the lungs are generally exempt from pathological changes, it is now known that both the pulmonary and the bronchial arteries may be involved. Indeed, the pulmonary findings may predominate clinically. A dry hacking cough, marked eosinophilia, and diffuse and fleeting infiltrations together with other evidences of wide-spread vascular involvement suggest the true nature of the disease process.

Dermatomyositis may severely damage the diaphragm and other respiratory muscles, thereby producing dyspnea and even asphyxia. Pulmonary fibrosis, with or without cystic formation, is often produced by scleroderma. In these cases, cough productive of a white mucoid material is to be expected. On the other hand, respiratory movements may be embarrassed by the thickening and immobilization of the leathery skin of the chest wall.

Damage to the alveolar capillaries in patients with disseminated lupus leads to pleurisy and to transient and repeated pulmonary consolidations. Clinically and pathologically these are similar to those infiltrations seen in periarteritis nodosa.

Neurological Manifestations

Approximately one-half of those afflicted by periarteritis nodosa have prominent symptoms of polyneuritis. Single nerves are usually involved, frequently in an asymmetrical pattern. The commonest symptoms are hyperesthesias, numbness, and tingling. Symptoms of central nervous system ischemia occur but are far less common.

In dermatomyositis one may encounter hyperesthesia, numbness of hands and feet, and foot drop. Although muscle involvement will often depress deep tendon reflexes, supratentorial changes may produce increased tendon reflexes and other

pyramidal signs. These neurological signs and symptoms are particularly pronounced in the rare form of the disease termed neurodermatomyositis.

In 50% of those with scleroderma, Raynaud's syndrome and other signs of vasomotor instability are present. Recurrent episodes of cold and cyanotic extremities may appear for years before the skin changes become apparent.

Epileptiform seizures may occur as a prodromal symptom of disseminated lupus, or they may first appear during the active phase of the disease.

Summary

In closing, I will attempt to simplify the multitudinous signs and symptoms of these diseases by listing only their most important diagnostic points. I have already indicated that fever, together with skin and joint symptoms, is found in some degree in each malady.

Polyarteritis or periarteritis nodosa:
- Prolonged and bewildering complex of symptomatology in unrelated organs
- Males, 2:1; average age of onset 35 years
- Fever, weakness, prostration, abdominal pain
- Leucocytosis, marked eosinophilia
- Polyneuritis
- Progressive hypertension (not found in the other groups)

Dermatomyositis:
- Commoner in children
- Periorbital or diffuse facial edema, "heliotrope lids"
- Muscular tenderness and weakness in shoulder girdle and upper arm in adults or in leg muscles in children

Generalized scleroderma:
- Females, 2:1; average age 40 years
- Cyanotic extremities and Raynaud's phenomena
- Mild stiffening of the jaws, dysphagia
- Peculiar hardening, induration, and rigidity of arm and facial skin "Claw hand"

Disseminated lupus erythematosus:
- Females, 4:1; between puberty and menopause;
- Prolonged irregular fever
- Microscopic hematuria together with mild albuminuria and leucopenia, superimposed on symptoms of rheumatoid arthritis

Depression of bone marrow function, L.E. cells

"Butterfly" rash on bridge of nose.

30 West 59th St. (19).

Pathology of Diffuse Collagen Diseases

Current Concepts

LORENZ E. ZIMMERMAN, M.D.
Washington, D. C.

Mr. President, Members of the Congress, I must confess that when I was invited to participate in this Panel, I was very much surprised that the subject of "collagen diseases" should find its place along with glaucoma, intraocular tumors, retinal detachment, and other more conventional ophthalmologic subjects assigned for panel discussions. At first I thought you might be interested in seeing some of the pathologic alterations that occur within the ocular tissues in the diffuse collagen diseases. But, on reconsideration, particularly in light of the rapid advances and wealth of publications during the last few years, I thought it would be more profitable to summarize some of our knowledge of the connective tissues and to discuss the present concept of their systemic disorders which have been set apart as "diffuse collagen diseases." What I propose to say is not based on investigations of my own but upon a review of some of the current literature. Although it is impossible for any one person to keep pace with the rapid advances that have been made and which have resulted in a voluminous literature, the interested reader is fortunate in having a number of excellent symposia available for study. I refer particularly to the five Josiah Macy, Jr. Foundation Conferences [32] on connective tissues, held in New York, 1950-1954; to the discussion on the nature and structure of collagen [38] held at King's College, London, in March, 1953, and to the book on "Connective Tissue in Health and Disease" edited by Asboe-Hansen and written by a select group of international authorities.[2] For those who are interested primarily in the collagen diseases, there are concise reviews that summarize and integrate the advances made in anatomy, physiology, and chemistry for our modern concept of the pathologic anatomy and physiology of these disorders.*

The term "collagen diseases" has unfortunately become a multiheaded monstrosity. I know that many ophthalmologists share with their other medical colleagues the mixed feelings of blissful ignorance, uncertainty, confusion, fear, and contempt engendered by its ever increasing use and abuse. To some internists and pathologists it has become nothing more than a convenient waste basket comparable to the equally meaningless term "uveitis" as employed by many ophthalmologists. To cautious investigators, however, the concept of the "collagen diseases" continues to be useful for pathogenetic synthesis and analysis.

Since the days of Bichat there has gradually evolved an impression of the connective tissue as an organ system capable of responding systemically as well

Received for publication March 18, 1956.

Read in the Symposium on Collagen Diseases at the Fifth Pan-American Congress of Ophthalmology, Santiago, Chile, Jan. 9, 1956.

From the Central Laboratory for Anatomical Pathology and Research, Veterans Administration, and the Armed Forces Institute of Pathology.

* References 8, 13, 14, 23, and 43.

as locally to injurious agents.[43] I should like to state at the outset, and I shall return to this later, that our increasing interest in the anatomy and physiology of connective tissue, of which collagen is but one element, will find its greatest reward for ophthalmology not in the systemic connective tissue diseases but in the more localized disturbances affecting such highly specialized connective tissue structures as the cornea and vitreous. Our current emphasis on the concept of the interstitial tissues as something more than mere supporting structures stems primarily from Klinge's writings on rheumatic fever and the contributions of Klemperer, Pollack, and Baehr in 1941 and 1942.† Klemperer and his associates, in coining the term "diffuse collagen disease," emphasized two essential features of disseminated lupus erythematosus and generalized scleroderma. They called attention to (1) the conspicuous morphologic and physiologic alterations of the colloidal intercellular components of the connective tissues and (2) the participation of the entire connective tissue system in these alterations. It is perhaps unfortunate that the term "collagen diseases" was employed, since the name itself apparently has been responsible for some semantic difficulties. On the one hand, it has seemingly singled out one component, and probably not the most important one, of the connective tissue elements.‡ Conversely, it has probably been responsible for the inclusion of many purely localized lesions involving collagen as examples of "collagen diseases" by writers who have overlooked or disregarded the essential requirement that the process involve the connective tissue in its entirety. Ehrich's[14] suggestion that we speak of "systemic diseases of the connective tissue" might tend to overcome some of these difficulties.

Today most authorities are in agreement that there is good reason to expand the group of diffuse collagen diseases to include rheumatic fever, rheumatoid arthritis, disseminated lupus erythematosus, generalized scleroderma, dermatomyositis, polyarteritis, and serum sickness. The hasty addition of many other generalized and localized diseases to the group, simply because they present isolated features in common with the accepted members, is only to be condemned. Klemperer emphasized the fact that it is erroneous to identify one disease with another merely because they share the site of anatomical alterations. Although originally grouped on the basis of anatomical features, the collagen diseases are now characterized also by certain biochemical and serologic alterations which they possess in common.[14]

Since the concept of these disorders is predicated upon a knowledge of the anatomy and physiology of the connective tissue, we should review briefly some of the current ideas about this ubiquitous organ. Essentially it consists of mesenchymal cells and a variety of acellular fibers suspended together in a hydrated gel, the interstitial tissue (Table 1). Fibroblasts, mast cells, histiocytes, plasma cells, and lymphocytes are the principal cellular elements. The fibers are collagenous, reticular, and elastic.

Collagen fibers are wavy birefringent bundles of unbranched acidophilic fibrils cemented tightly together in a polysaccharide matrix. They are characterized chemically by their low content of aromatic amino acids, their high content of proline, hydroxyproline, and aminoacetic acid (glycine), and their enzymatic

† References 26 and 27.

‡ Klemperer and co-workers[26] left no doubt that they were referring to the complete intercellular substance, but they employed the word "collagen" in a nonspecific sense for brevity's sake.

TABLE 1.—*Essential Components of Connective Tissue*

Cellular	
	1. Fibroblasts
	2. Reticulum cells
	3. Mast cells
	4. Plasma cells, lymphocytes, etc.
Fibrillar	
	1. Collagens
	2. Reticulins
	3. Elastins
Interstitial	
	1. Ground substances
	2. Cement substances
	3. Tissue fluids

hydrolysis by collagenase. Physically they are distinguished by a particular wide-angle x-ray diffraction pattern and by periodic banding at 640 A. intervals.

Reticular fibers are fine, branching and anastamosing, argyrophilic deposits which are present in the basement membranes of epithelia, the framework of lymphoid organs, and the boundary tissue about muscle fibers, fat cells, and blood vessels. They are not birefringent but are stained intensely by the periodic acid-Schiff reaction. After treatment with sulfuric acid, reticular fibers become metachromatic, while collagen remains orthochromatic.[28] Reticular fibers are apparently not significantly different morphologically from the individual fibrils of immature collagen. Furthermore, histoenzymatic studies reveal identical reactions of collagen and reticulin: Both are lysed by pepsin and collagenase but resist trypsin and testicular hyaluronidase.[28] Nevertheless, there is reason to believe that an important chemical difference exists between reticulin and collagen, for immunologic studies have revealed them to be distinct. With the aid of fluorescent antiglomerulus globulin, Cruickshank and Hill found that a common antigen is present in the basement membranes and reticulin of man and rat.[10] At no time, however, did this histoimmunologic method reveal the antigen to be present in collagen fibers. It has been concluded that while the fibrillar component of reticulin is almost certainly a variety of collagen, the important histologic, chemical, and immunologic dissimilarities between reticular and collagen fibers are related to differences in the character and amount of ground substance and to the relationship of fibers to polysaccharides within the ground substance.§

Although the nature of elastic fibers has been the subject of less intensive investigation, there is general agreement that their physical characteristics and chemical composition differ from those of collagen and reticulin.[37] Elastic fibers are refractile branching structures which vary considerably in size, shape, distribution, and staining characteristics according to their anatomical site. Although refractory to most staining procedures, elastin has an affinity for resorcin-fuchsin and orcein, and also Schiff's reagent after treatment with periodic acid. It is remarkably insoluble, remaining after everything else in the tissue has been removed by dilute acid or alkali. The amino acid content of elastin differs from that of collagen and is not the same in the young as in the old. The increased amount of aspartic and glutamic acids in the aortas of older persons may account for the calcium-affinity of aging elastic tissue.[30] Elastin resists pure trypsin and pepsin, but it is lysed by a newly discovered enzyme obtained from the pancreas, elastase. Physically the elastic fiber is conceived to be an infinite number of generations of paired helically-coiled fibrils. One fiber treated with elastase splits

§ References 4, 10, 14, 16, 21, 28, and 40-42.

into two coiled fibrils, each of which gives rise to two more coiled fibrils, and so on until the fibrils can no longer be seen with the light microscope. Results obtained by electron microscopy have been subject to question, since the preparations were not pure and since helically-coiled fibrils almost identical with those of elastic fibrils may be observed in phage-treated bacteria and in calcium soaps.[30]

So much for the fibrillar elements; what about the interstitial components? Connective tissue contains an abundance of mucopolysaccharides which may be lumped into two large groups with reference to the presence or absence of sulfate. The highly hydrated sulfate-free hyaluronic acids are concentrated in the ground substance between the formed elements. In some tissues, such as the vitreous, only these nonsulfated polysaccharides are present. Although they function as lubricants and shock absorbers,[34] they are not metabolically inert. Hyaluronic acid has a rapid turnover [12] and probably is a most important factor in the exchange of fluids and electrolytes through the hematoparenchymal barrier. The sulfated polysaccharides apparently form the cement substance within collagen and cartilage.‖ They also function in the binding of cations in a manner comparable to synthetic ion-exchange resins.[34] The tissue fluids are conceived to be homogeneously coextensive with the mucopolysaccharides of the ground substance as a result of being incorporated into the gel state of the latter.[15] Although the precise origin of these acellular elements of connective tissue is not established to the satisfaction of all investigators, it is generally believed that the mucopolysaccharides are secreted by mesenchymal cells and that collagen develops extracellularly but under the influence of fibroblastic activity.

The physiology of connective tissue is extremely complex, and, even if I were qualified to discuss the newer knowledge that is accumulating in this field, time would not permit my doing so. Furthermore, there is a lack of agreement upon many basic aspects of connective tissue structure and function.[37] Excellent reviews may be found in recent publications.¶ I should like to emphasize the complexity of connective tissue function, which depends not only upon fibroblastic activity but also upon a satisfactory supply of building stones from the blood and such physiologic regulators as enzymes, enzyme-inhibitors, vitamins, and hormones. The hyaluronidases, specific and nonspecific antihyaluronidases, collagenase, and heparin are all examples of enzymes and enzyme-inhibitors which may play important roles in the function of connective tissue. Ascorbic acid is the most important vitamin. Among the hormones, testosterone, estrogens, gonadotropins, corticotropin (ACTH), thyrotropic hormone, desoxycorticosterone acetate (DOCA), and cortisone have definite effects upon connective tissue. These various factors operate through many different pathways, some known, many unknown. All of this is mentioned to emphasize the point that the tissue we are discussing is much more than just a "connecting" substance. It is a barrier which must be crossed by water, electrolytes, nutrients, and metabolites passing between the blood stream and parenchymatous tissue cells. It is living matter in a state of constant flux, capable of great changes brought about by alterations in the state of polymerization of polysaccharides, movement of bound water, and activity of its cellular elements. All of these biochemical and biophysical phenomena must be under neurogenic control; witness the dramatically sudden development of exophthalmos following psychic trauma.

‖ References 14 and 34.

¶ References 3, 12, 14, 19, 33, 44, and 46.

Now let us pass from the anatomy and physiology of connective tissue to its pathology. The principal systemic alterations in connective tissue are degenerative and proliferative. The former includes mucoid, fibrinoid, amyloid, and paramyloid changes; the latter, fibroblastic activity, sclerosis, and hyalinization. Mucoid degeneration is due to an accumulation of depolymerized acid mucopolysaccharides which give the tissue a pale basophilic fibrillar appearance when seen in routine histologic sections. Although this type of degenerative change is observed in the "collagen diseases," it is also seen under many other circumstances and is entirely nonspecific. Fibrinoid degeneration refers to the formation in connective tissue of smudgy, often refractile, acidophilic fibrillar band-like deposits resembling fibrin. This fibrinoid material, which is distinct from both collagen and fibrin, is believed to represent a precipitation of proteinaceous material within the ground substance of connective tissue. The source and chemical characteristics of this protein-polysaccharide precipitate are different in the various conditions in which it may be observed. Fibrinoid degeneration is therefore as nonspecific as mucoid degeneration, and the two are often observed together. Amyloid and paramyloid are homogeneous, structureless hyaline deposits in the ground substance and basement membranes. They are believed to be mucopolysaccharide-protein complexes differing somewhat from one another in physical and chemical characteristics. Ehrich, Teilum, and others suggested that amyloidosis and paramyloidosis are due to faulty production of antibody proteins (γ-globulins) by dysfunctioning mesenchymal cells. Proliferative changes in connective tissue involve fibroblasts, their undifferentiated predecessors, perithelial and endothelial cells, and, to varying degrees, lymphocytes, plasma cells, and macrophages. After fibroblastic activity much collagen may be deposited, a condition we call fibrosis or sclerosis. When the collagen fibers are transformed into thick homogeneous acidophilic bands, we speak of hyalinization or hyalinosis. Associated with these proliferative responses are inflammatory reactions to the basic connective tissue damage.

In the various collagen diseases, one observes all of the degenerative, proliferative, and inflammatory changes occurring in varying degree, in varying intensity, and in varying proportion. All begin with mucoid degeneration, which is taken as evidence of disturbed fibroblastic activity. In the severer or more acute cases mucoid degeneration is associated with or followed by fibrinoid degeneration. In the less severe, fibroblastic proliferation may dominate the histologic picture, while in chronic cases there is fibrosis or hyalinization. Histologic evidence of amyloidosis may be found with great frequency in rheumatoid arthritis [44]; paramyloidosis is encountered in disseminated lupus.[14] Although these alterations, observed to varying degrees and in varying proportions, are characteristic of the collagen diseases, it must be emphasized that not one is in any way specific or pathognomonic. These are the morphologic similarities of the collagen diseases; what about their differences?

Were it not for characteristic clinical and pathological differences, we should not be faced with the necessity of considering so many different entities. It is upon these dissimilarities that our differential diagnosis is based,# and presumably these dissimilarities indicate etiologic differences. The morphologic differences include not only a varying ratio of degenerative to proliferative changes but also, more importantly, variations in specific organ involvement, type and

References 5, 13, 14, and 22.

size of vascular lesions, and the degree of inflammatory reaction. We need not go into the specific details of these differences. For those of you who wish to review them, I will refer you to the excellent summarization by Ehrich in 1952.

Just as some of the morphologic changes are common to the collagen diseases, so are some of the chemical and serologic alterations.[14] Serum albumin and the collagenase inhibitor are depressed, suggesting liver damage. The elevation of other serum proteins, the serum mucopolysaccharides, and the nonspecific hyaluronidase inhibitor may be the result of diffuse mesenchymal injury. Usually there is a rise in γ-globulins. Presumably this is always due to specific antibodies. The generalized increase in plasma cells and lymphocytes observed in the collagen diseases is taken as evidence that these cells produce the specific γ-globulins.

Serologic and chemical differences may be characteristic enough to be valuable aids to clinical differentiation: high titers of antistreptococcal antibodies in rheumatic fever, strong nonspecific agglutination reaction in disseminated lupus, and specific precipitins in serum sickness.[14]

The introduction of the concept of a group of diffuse collagen diseases has resulted in a number of worthwhile accomplishments: rebirth of interest in the connective tissues as a system; intensification of basic research into the morphology, biochemistry, biophysics, serology, and pathology of all elements of connective tissue, and emphasis on thinking pathogenetically as well as etiologically. The latter distinction is often overlooked by many physicians, who use the terms pathogenesis and etiology interchangeably. The beneficial effect of corticotropin and cortisone in many cases of diffuse collagen disease has sometimes led to the assumption of a common etiology. This is no more reasonable than would be the assumption that all uveal tract diseases responding to mydriatics are produced by the same etiologic factors.

Ehrich has developed an interesting working hypothesis to explain the pathogenesis of these diseases, even though their specific etiologies may differ greatly. He has suggested that the diffuse alteration in the connective tissues results from injury by abnormal γ-globulins produced by plasma cells. These systemic diseases of the connective tissues might, therefore, be designated as due to dysgammaglobulinemias in order to separate them from diffuse connective tissue disorders due to avitaminosis (scurvy), hormone imbalance (myxedema), hereditary factors (arachnodactyly), and other disturbances in which the cause of the connective tissue damage is not a γ-globulin. This concept sidesteps the problem of etiology very nicely, for nothing is said about the specific stimulus which excites the production of abnormal γ-globulins by plasma cells. It also does not preclude the possibility that for each member of the "diffuse collagen diseases" there might be more than one etiology. Ehrich's thesis is also attractive for explaining why

TABLE 2.—*Classification of Connective Tissue Diseases*

I. Localized: due to
   A. Mechanical trauma (ex. keloid)
   B. Actinic trauma (ex. pterygium)
   C. Infection (ex. erysipelas)
   D. Allergy (ex. angioneurotic edema)
   E. Disturbed hydrodynamics (ex. corneal edema in glaucoma)
   etc.

II. Systemic: due to
   A. Avitaminosis (ex. scurvy)
   B. Metabolic abnormalities (ex. gout)
   C. Hormonal imbalance (ex. myxedema)
   D. Hereditary factors (ex. arachnodactyly)
   E. Dysgammaglobulinemias (ex. lupus erythematosus)
   etc.

purely localized connective tissue disorders should not be lumped with the collagen diseases. We can then arrive at a rough classification of connective tissue diseases that places the "diffuse collagen diseases" in their proper perspective (Table 2).

Before closing I should like to make a few remarks relative to connective tissue disorders in ophthalmology. Here the purely localized types of connective tissue damage are undoubtedly of vastly greater importance than are the diffuse collagen diseases. The recent advances that have been made as a result of the upsurge in interest in connective tissue should lead eventually to a better understanding of diseases of the cornea, vitreous, sclera, and orbit. These structures are largely mesenchymal, and not only do they contain an abundance of mucopolysaccharides but they are composed of distinctive compounds. The cornea, for example, contains a substance (keratosulfate) which accounts for half the total mucopolysaccharide fraction of the cornea. This particular compound has the distinction of being the only mucopolysaccharide of animal origin which does not contain a uronic acid. It has not yet been demonstrated in any tissue other than the cornea.[34] It is, of course, very tempting to associate the presence of this unusual ground substance with the remarkable ability of the cornea to remain deturgescent even though in vitro it has a greater capacity to soak up water than any other nonfetal tissue.[7]

Undoubtedly the advances being made in our understanding of the long-neglected connective tissues will yield great dividends to ophthalmology. This will be due not to any assumed importance of ocular lesions in the diffuse collagen diseases but because of a better understanding of the pathologic physiology in diseases of the cornea, chronic simple glaucoma, degenerations of the vitreous, malignant glaucoma, retinal separation, endocrinopathic exophthalmos, and pseudotumors of the orbit.

REFERENCES

1. Altshuler, C. H., and Angevine, D. M.: The Pathology of Connective Tissue, in Asboe-Hansen, G.: Connective Tissue in Health and Disease, Copenhagen, Ejnar Munksgaards Forlag, 1954, pp. 178-195.

2. Asboe-Hansen, G.: Connective Tissue in Health and Disease, Copenhagen, Ejnar Munksgaards Forlag, 1954.

3. Asboe-Hansen, G.: Hormonal Effects on Connective Tissues, in Transactions of the Fifth Macy Foundation Conference on Connective Tissues, Feb. 8-10, 1954, edited by C. Ragan, New York, Josiah Macy, Jr. Foundation Publications, 1954, pp. 123-182.

4. Banfield, W. G.: Aging of Connective Tissue, in Asboe-Hansen, G.: Connective Tissue in Health and Disease, Copenhagen, Ejnar Munksgaards Forlag, 1954, pp. 151-158.

5. Banks, B. M.: Is There a Common Denominator in Scleroderma, Dermatomyositis, Disseminated Lupus Erythematosus, Libman-Sacks Syndrome and Polyarteritis nodosa? New England J. Med. 225:433-444, 1941.

6. Bárány, E. H., and Scotchbrook, S.: Influence of Testicular Hyaluronidase on the Resistance to Flow Through the Angle of the Anterior Chamber, Acta physiol. scandinav. 30:240-248, 1954.

7. Cogan, D. G.: Applied Anatomy and Physiology of Cornea, Tr. Am. Acad. Ophth. 55:329-359, 1951.

8. Colomb, D.: Des Altérations du tissu conjonctif en dermatologie: Des collagénoses aux collagénomes, Thesis, Lyon, 1953.

9. Cowan, P. M.; North, A. C. T., and Randall, J. T.: High-Angle X-Ray Diffraction of Collagen Fibres, in Randall, J. T.: Nature and Structure of Collagen, New York, Academic Press, Inc., 1953, pp. 241-249.

10. Cruickshank, B., and Hill, A. G. S.: Histochemical Identification of a Connective Tis-

sue Antigen, in Randall, J. T.: Nature and Structure of Collagen, New York, Academic Press, Inc., 1953, pp. 27-32.

11. Dische, Z., and Zelmanes, G.: Polysaccharides of the Vitreous Fibers, A. M. A. Arch. Ophth. 54:528-538, 1955.

12. Dorfman, A.: Metabolism of the Mucopolysaccharides of Connective Tissue, in Asboe-Hansen, G.: Connective Tissue in Health and Disease, Copenhagen, Ejnar Munksgaards Forlag, 1954, pp. 81-96.

13. Duff, G. L.: The Diffuse Collagen Diseases: A Morphological Correlation, Canad. M. A. J. 58:317-325, 1948.

14. Ehrich, W. E.: Nature of Collagen Diseases, Am. Heart J. 43:121-156, 1952.

15. Gersh, I.: Some Functional Considerations of Ground Substance of Connective Tissues, in Transactions of the Second Macy Foundation Conference on Connective Tissue, May 24-25, 1951, edited by C. Ragan, New York, Josiah Macy, Jr. Foundation Publications, 1951, pp. 11-44.

16. Glynn, L. E.: Discussion A, in Randall, J. T.: Nature and Structure of Collagen, New York, Academic Press, Inc., 1953, p. 46.

17. Godtfredsen, E.: Investigations into Hyaluronic Acid and Hyaluronidase in the Subretinal Fluid in Retinal Detachment, Partly Due to Ruptures and Partly Secondary to Malignant Choroidal Melanoma: Preliminary Report Suggesting a New Hypothesis Concerning the Pathogenesis of Retinal Detachment, Brit. J. Ophth. 33:721-732, 1949.

18. Irvine, S. R.; Brunish, R., and Rowen, J. W.: Studies on the Vitreous Humor: Further Observations on Sequelae and Treatment of Prolapsed Vitreous; Properties of Vitreous Dependent upon the Hyaluronic-Acid Content, Am. J. Ophth. 39:100-108 (April, Pt. 2) 1955.

19. Iversen, K.: Hormonal Influence on Connective Tissue, in Asboe-Hansen, G.: Connective Tissue in Health and Disease, Copenhagen, Ejnar Munksgaards Forlag, 1954, pp. 130-150.

20. Jackson, S. F.: Fibrogenesis in Vivo and in Vitro, in Randall, J. T.: Nature and Structure of Collagen, New York, Academic Press, Inc., 1953, pp. 140-151.

21. Jacobson, W.: Histological Survey of the Normal Connective Tissue and Its Derivatives, in Randall, J. T.: Nature and Structure of Collagen, New York, Academic Press, Inc., 1953, pp. 6-13.

22. Keefer C. S.: Collagen Diseases: Pathology and Clinical Course, M. Clin. North America 35:1267-1281, 1951.

23. Klemperer, P.: The Concept of Collagen Diseases, Am. J. Path. 26:505-519, 1950.

24. Klemperer, P.: Pathology of Systemic Lupus Erythematosus, in McManus, J. F. A.: Progress in Fundamental Medicine, Philadelphia, Lea & Febiger, 1952, Ch. 3, pp. 51-68.

25. Klemperer, P.: General Considerations on Collagen Diseases, in Asboe-Hansen, G.: Connective Tissue in Health and Disease, Copenhagen, Ejnar Munksgaards Forlag, 1954, pp. 251-262.

26. Klemperer, P.; Pollack, A. D., and Baehr, G.: Pathology of Disseminated Lupus Erythematosus, Arch. Path. 32:569-631, 1941.

27. Klemperer, P.; Pollack, A. D., and Baehr, G.: Diffuse Collagen Disease: Acute Disseminated Lupus Erythematosus and Diffuse Scleroderma, J. A. M. A. 119:331-332, 1942.

28. Kramer, H., and Little, K.: Nature of Reticulin, in Randall, J. T.: Nature and Structure of Collagen, New York, Academic Press, Inc., 1953, pp. 33-43.

29. Kulonen, E.: Recent Advances in the Chemistry of Collagen, in Asboe-Hansen, G.: Connective Tissue in Health and Disease, Copenhagen, Ejnar Munksgaards Forlag, 1954, pp. 70-80.

30. Lansing, A. I.: Chemical Morphology of Elastic Fibers, in Transactions of the Second Macy Foundation Conference on Connective Tissue, May 24-25, 1951, edited by C. Ragan, New York, Josiah Macy, Jr. Foundation Publications, 1951, pp. 45-85.

31. Lillie, R. D.: Connective Tissue Staining, in Transactions of the Third Macy Foundation Conference on Connective Tissues, Feb. 14-15, 1952, edited by C. Ragan, New York, Josiah Macy, Jr. Foundation Publications, 1952, pp. 11-37.

32. Transactions of Annual Conferences on Connective Tissues, edited by Charles Ragan, New York, Josiah Macy, Jr. Foundation Publications, 1950-1954.

33. Mathews, M. B., and Dorfman, A.: Inhibition of Hyaluronidase, in Asboe-Hansen, G.: Connective Tissue in Health and Disease, Copenhagen, Ejnar Munksgaards Forlag, 1954, pp. 112-129.

34. Meyer, K.: The Chemistry of the Ground Substances of Connective Tissue, in Asboe-Hansen, G.: Connective Tissue in Health and Disease, Copenhagen, Ejnar Munksgaards Forlag, 1954, pp. 54-69.

35. Moore, J. E., and Lutz, W. B.: The Natural History of Systemic Lupus Erythematosus: An Approach to Its Study Through Chronic Biologic False Positive Reactors, J. Chron. Dis. 1:297-316, 1955.

36. Pirie, A.: Ox Vitreous Humour: 2. Hyaluronic Acid Relationships, Brit. J. Ophth. 33:271-283, 1949.

37. Ragan, C., and others: General Areas of Agreement Reached in this Conference Group, in Transactions of the Fifth Macy Foundation Conference on Connective Tissues, Feb. 18-20, 1953, edited by C. Ragan, New York, Josiah Macy, Jr. Foundation Publications, 1953, pp. 17-46.

38. Randall, J. T., Editor: Nature and Structure of Collagen, New York, Academic Press, Inc., 1953.

39. Randall, J. T.: Physical and Chemical Problems of Fibre Formation and Structure, in Randall, J. T.: Nature and Structure of Collagen, New York, Academic Press, Inc., 1953, pp. 232-240.

40. Robb-Smith, A. H. T.: The Nature of Reticulin, in Transactions of the Third Macy Foundation Conference on Connective Tissues, Feb. 14-15, 1952, edited by C. Ragan, New York, Josiah Macy, Jr. Foundation Publications, 1952, pp. 92-116.

41. Robb-Smith, A. H. T.: Significance of Collagenase, in Randall, J. T.: Nature and Structure of Collagen, New York, Academic Press, Inc., 1953, pp. 14-26.

42. Robb-Smith, A. H. T.: Normal Morphology and Morphogenesis of Connective Tissue, in Asboe-Hansen, G.: Connective Tissue in Health and Disease, Copenhagen, Ejnar Munksgaards Forlag, 1954, pp. 15-30.

43. Robb-Smith, A. H.: The Concept of the Collagen Diseases, Practitioner 173:117-124, 1954.

44. Teilum, G.: Cortisone, Ascorbic Acid, and Changes in the Reticuloendothelial System, in Asboe-Hansen, G.: Connective Tissue in Health and Disease, Copenhagen, Ejnar Munksgaards Forlag, 1954, pp. 196-207.

45. Wyckoff, R. W. G.: The Fine Structure of Connective Tissues, in Transactions of the Third Macy Foundation Conference on Connective Tissues, Feb. 14-15, 1952, edited by C. Ragan, New York, Josiah Macy, Jr. Foundation Publications, 1952, pp. 38-91.

46. Zweifach, B. W.: The Exchange of Materials Between Blood Vessels and Lymph Compartments, in Transactions of the Fifth Macy Foundation Conference on Connective Tissues, Feb. 8-10, 1954, edited by C. Ragan, New York, Josiah Macy, Jr. Foundation Publications, 1954, pp. 38-77.

Ocular Manifestations of Collagen Diseases

A. EDWARD MAUMENEE, M.D.
Baltimore

The term "collagen diseases" was first used by Klemperer [1] to stress the importance of the connective tissue reaction in, and to stimulate investigation of, a group of diseases with heterologous clinical manifestations. The most obvious pathologic feature which is found in each of these diseases is a fibrinoid degeneration of the connective tissue. Neumann,[2] in 1880, thought that this type of reaction was due to a transformation of the collagen fibers into fibrin-like material. Recently, however, Altshuler and Angevine [3] have concluded that the homogenous ground substance, and not the collagen fiber, is the anatomic site of the connective tissue alteration and that the fibrinoid deposits are a result of a precipitation of mucopolysaccharides by the action of a basic protein. Thus, it appears that the term "systemic diseases of the connective tissue" or "dysgammaglobulinemia" is a more appropriate designation than collagen diseases.[4] However, since the term collagen diseases is well established, and since it is not intended to imply any specific alteration, there is no need to make a change in the terminology at this time.

The concept that these reactions are due to a hypersensitive response has likewise become more generally used than was originally intended. This occurred because Klinge [5] noted fibrinoid changes in the connective tissue of rabbits made hypersensitive to foreign proteins, and other workers assumed then that such alteration always meant hypersensitivity. Klemperer [6] points out that fibrinoid degeneration can occur in a wide variety of conditions, such as peptic ulcers, pancreatic necrosis, acute bacterial infections, animals made hypertensive by the Goldblatt mechanism, repeated injections of epinephrine, or even simple squeezing of the skin of the rat. He does not deny that many of the illnesses originally included under the term collagen diseases are not related to a hypersensitive reaction. He warns, however, that just because one of these conditions is found to be caused by an allergic response does not necessarily mean that a similar etiology will be found in the other diseases. He also points out the danger of using the name collagen diseases as a catch-all for all maladies with puzzling clinical and anatomical features.

Since the term collagen diseases was originally used to include a group of conditions of unknown etiology, it is difficult to decide which eye lesions should be included in a discussion of the ocular manifestations of these conditions. This decision will be by-passed to some extent by describing most of the ocular lesions

Received for publication April 6, 1956.

From the Wilmer Ophthalmological Institute of the Johns Hopkins Hospital and University.

Read in the Symposium on Collagen Diseases at the Fifth Pan-American Congress of Ophthalmology, Santiago, Chile, Jan. 9, 1956.

which have been previously included in the excellent reviews of Stillerman [7] and Vail.[8]

Collagen Diseases

The systemic diseases most frequently included under the term collagen diseases are lupus erythematosus disseminatus, periarteritis nodosa, dermatomyositis, generalized scleroderma, serum sickness, rheumatic fever, and rheumatoid arthritis. In order to avoid a separate description of the ocular lesions in these conditions, the structures most frequently involved in each disease are listed in the accompanying Table.

Ocular Structures Involved in the Collagen Diseases

	L.E.D.*	P.A.N.†	Dermato-myositis	Sclero-derma	Serum Sickness	Rheumatic Fever	Rheumatic Arthritis
Lids	+	+	+	+	+		+
Conjunctiva	+	+			+		
Sclera		+	+				+
Cornea		+			+		+
Lens				+			
Retina	+	+	+		+		
Uvea	+	+	+			?rarely	+
Optic Nerve	+	+					
Orbit		+	+				
C.N.S.‡	+	+	+		+		

*Lupus erythematosus disseminatus.
†Periarteritis nodosa.
‡Central nervous system.

Retinal, optic nerve, and choroidal involvement are the most constant findings in lupus erythematosus disseminatus, periarteritis nodosa, dermatomyositis, and serum sickness. Tumulty [9] has observed such changes in about 25% of patients with acute lupus erythematosus disseminatus. These findings consist of cytoid bodies (cotton-wool exudates) and flame-shaped hemorrhages in the nerve fiber layer of the retina, edema of the nerve head, and transudates and leucocytic-cell infiltration in the choroid (septic choroiditis). All of these conditions are probably manifestations of injury and increased permeability of the capillaries. The exact nature of cytoid bodies is not known, but they probably represent either swollen degenerating neuroglia fibers or transudates [10] (Fig. 1). It is possible that they may be similar to the transudates in the connective tissue elsewhere in the body in these diseases.

The typical retinal picture in the early stages of these conditions consists of a varying number of cotton-wool spots in the posterior part of the fundus with a few flame-shaped hemorrhages and occasionally slight papilledema. The retinal vessels appear normal (Fig. 2). These lesions usually occur during an exacerbation of the general disease and may clear as the patient's systemic manifestations

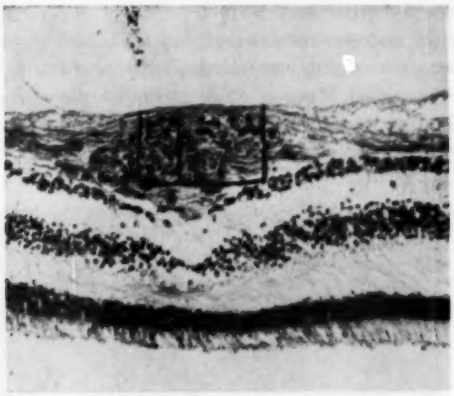


Fig. 1.—Cytoid body in nerve fiber layer of retina, showing eosinophilic-staining material representing either transudates or swollen neuroglia fibers.

Fig. 2.—Cytoid bodies (cotton-wool exudates) in retina. Blood vessels normal.

improve. As the illness progresses, if the patient develops hypertension, retinal vascular lesions may become evident. The early retinal changes of this type are not pathognomonic for the collagen diseases. However, if numerous cytoid bodies are present in a patient whose fundus is otherwise normal and who has several of the following symptoms—a prolonged low-grade fever, arthritis, leucopenia, anemia, and cardiac and kidney involvement—then one of the above listed diseases should be suspected.

Baehr and Pollack [11] have stated that in the early stages of rheumatic fever, rheumatoid arthritis, and disseminated lupus erythematosus the differential diagnosis is sometimes difficult. In these instances the presence of fundus changes sharply differentiates lupus erythematosus from the other two conditions, for they do not occur in rheumatic fever or rheumatoid arthritis.

A nongranulomatous type of iritis has been occasionally observed in periarteritis nodosa and dermatomyositis. Retinal perivasculitis and occlusion of the central retinal artery has also been noted in periarteritis nodosa.

Mild to severe episcleritis and nodular scleritis are complications which accompany periarteritis nodosa, rheumatoid arthritis, and dermatomyositis. Especially in the former two conditions scleromalacia perforans, necrotizing scleritis with keratitis, and marginal Mooren's-like ulcers of the cornea have been noted. Microscopic examination of the necrotic scleral lesions shows evidence of fibrinoid degeneration and phagocytosis of the fragmented collagen fibers by monocytes (Fig. 3).

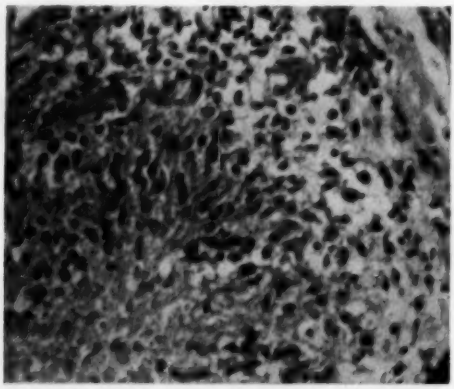


Fig. 3.—Biopsy specimen of scleral nodule from a case of scleritis, showing palisading of monocytes along scleral fibers, similar to the reaction in a rheumatic nodule.

The frequent association of nongranulomatous iridocyclitis and rheumatoid arthritis is of particular interest to ophthalmologists, for it offers the hope that if one of these trying problems can be solved the same answer can be applied to the other. The incidence of iritis in rheumatoid involvement of the spine, ankylosing spondylitis, is 8 to 10 times more frequent than in adult peripheral arthritis. When polyarthritis of the rheumatoid type occurs in children, the iritis is frequently accompanied by a band-shaped calcified opacity of the superficial cornea. The infrequent involvement of the choroid in uveitis associated with rheumatoid lesions suggests that there is some factor in the anterior chamber or iris which predisposes the iris vessels to damage. It is possible that the hyaluronidase or hyaluronic acid in the aqueous may be this factor.

The other lesions in the collagen diseases under discussion are rare. The lids may be involved in the skin changes of lupus erythematosus disseminatus and scleroderma, at times to a sufficient degree to produce an ectropion with secondary conjunctival changes. Lid, conjunctival, and orbital edema may occur, as listed in the Table. Cataracts are complications of scleroderma, and corneal edema is a rare complication in serum sickness. Ocular palsies, nystagmus, and transient hemianopsia or blindness may be present in lupus erythematosus disseminatus, periarteritis nodosa, and serum sickness as a result of central nervous system involvement.

Conditions Possibly Related to Collagen Diseases

Temporal arteritis or cranial arteritis occurs primarily in elderly patients, more frequently in females than in males. In 1949, Bruce [12] reviewed the 84 then-published cases of temporal arteritis and found ocular involvement in 40% of them. The commonest lesion was an ischemic retrobulbar neuritis. However,

arterial occlusion or retinal arterial spasm with temporary loss of vision can also occur. Severe headaches and marked tenderness of the temporal artery frequently precede the ocular changes by some weeks to months. The vessels which supply the head are usually involved in this condition, but vessels elsewhere in the body may also be affected. Some investigators think that this condition is closely related to, if not a form of, periarteritis nodosa.[13]

Behcet's syndrome consists of recurrent hypopyon iritis (Fig. 4), stomal and

Fig. 4.—Recurrent hypopyon iritis in patient with stomal and genital ulcers, erythema nodosum, and arthritis.

genital ulcers, and, at times, thrombophlebitis, erythema nodosum, pyodermia, and arthritis. Biopsies of the skin lesions show edema, diffuse cellular infiltration, and fibrinoid degeneration of the connective tissue similar to that in other collagen diseases.[14] The etiology of this lesion is not known, but an allergic factor has been suggested. Recurrent hypopyon iritis is an early ocular manifestation, but eyes that have had to be enucleated have shown diffuse destruction of the choroid and retina.

The following conditions have been included in discussions of collagen diseases by some authors because they are thought to be variants of erythema multiforme and related to Behcet's syndrome:

1. Reiter's syndrome, which consists of conjunctivitis, urethritis, arthritis, and occasionally iritis.

2. Stevens-Johnson's disease, or generalized cutaneous eruptions, stomatitis, and conjunctivitis.

3. Ectodermosis erosiva pluriorificialis, consisting of vascular lesions of the mucous membranes of the mouth, conjunctiva, genitalia, and skin of the extremities.

Conclusion

In spite of previous warnings, one is tempted to use the fallacious reasoning of *post hoc, ergo propter hoc*, and therefore conclude that any disease with fibrinoid changes in the tissues is due to an allergic reaction—also, that any ocular condition involving a tissue containing a large amount of collagen may be related to the so-called "collagen diseases." This has been even carried to

the extent of suggesting that myopia and keratoconus might be related to the diseases previously discussed.

It is reiterated that fibrinoid changes are the result of alterations in the mucoprotein ground substance and thus do not primarily involve collagen. Also, listing of these ocular lesions or any other disease under the heading of collagen diseases brings one no closer to the solution of their pathogenesis. It merely points out one morphological characteristic, fibrinoid degeneration of the connective tissue, which is found in these conditions. Thus, when a more specific change or etiology is discovered for one abnormality, that disease should be removed from the group of unknowns. Such findings in any one condition should be used as a stepping stone for investigations of the other lesions.

REFERENCES

1. Klemperer, P.; Pollack, A. D., and Baehr, G.: Diffuse Collagen Diseases, J. A. M. A. 119:331, 1942.

2. Neumann, E.: The Picrocarmine Stain and Its Application to the Study of Inflammation, Arch. mikr. Anat. 18:130, 1880.

3. Altshuler, C. H., and Angevine, D. M.: Histochemical Studies on the Pathogenesis of Fibrinoid, Am. J. Path. 25:1061, 1949.

4. Ehrich, W. E.: Nature of Collagen Diseases, Am. Heart J. 43:121, 1952.

5. Klinge, F.: Rheumatism, Ergebn. allg. Path. u. path. Anat. 27:1, 1933.

6. Klemperer, P.: General Considerations of Collagen Diseases, in Asboe-Hansen, G.: Connective Tissue in Health and Disease, Copenhagen, Ejnar Munksgaards Forlag, 1954.

7. Stillerman, M. L.: Ocular Aspects of Diffuse Collagen Diseases, in Sorsby, A.: Modern Trends in Ophthalmology, New York, Paul B. Hoeber, Inc., 1955.

8. Vail, D.: Diffuse Collagen Diseases with Ocular Complications, Tr. Ophth. Soc. U. Kingdom 72:155, 1952.

9. Tumulty, P. A.: Clinical Course of Systemic Lupus Erythematosus, J. A. M. A. 156:947, 1954.

10. Kiewe, P. L., and Hart, F. D.: Cytoid Bodies in the Retina in a Case of Malignant Disease, Tr. Ophth. Soc. U. Kingdom 71:309, 1951.

11. Baehr, G., and Pollack, A. D.: Disseminated Lupus Erythematosus and Diffuse Scleroderma, J. A. M. A. 134:1169, 1947.

12. Bruce, G. M.: Temporal Arteritis as a Cause of Blindness: Review of the Literature and Report of a Case, Tr. Am. Ophth. Soc. 47:300, 1949.

13. Zeek, P. M.: Periarteritis Nodosa: Critical Review, Am. J. Clin. Path. 22:777, 1952.

14. France, R.; Buchanan, R. N., Jr.; Wilson, M. W., and Sheldon, M. B., Jr.: Relapsing Iritis with Recurrent Ulcers of the Mouth and Genitalia (Behcet's Syndrome), Medicine 30:335, 1951.

Differential Diagnosis of Collagen Diseases

JACK S. GUYTON, M.D.
Detroit

This paper will concern itself with differentiating between the six syndromes typically classified as collagen diseases. Other diseases with manifestations common to these will not be differentiated, or even enumerated. Ocular manifestations will be omitted.

The approximate relative incidence of these six collagen diseases is as follows:

Rheumatoid arthritis - - -

There is no laboratory test pathognomonic of the collagen diseases as a group. The sedimentation rate is elevated during active phases of all, and it is a good index of the activity, although at times it remains elevated some while after clinical improvement begins. Also, serum globulin (alpha and beta) tends to be elevated more or less in proportion to the activity.

Rheumatoid Arthritis

Rheumatoid arthritis always involves joints, in more or less characteristic fashion. The arthritis is usually polyarticular and somewhat symmetrical, although occasionally only one joint is involved in the early stages. The proximal interphalangeal joints are especially likely to be involved. Swelling, tenderness, and limitation of motion of the involved joints tends to be of long duration and to cause permanent damage and deformity, exaggerated in cases of children. Weakness and atrophy of adjacent muscles is out of proportion to the joint involvement. Characteristic x-ray changes (decalcification, joint space narrowing, ankylosis) are not likely to appear for one or several years.

Rheumatoid arthritis may begin at any age, but it occurs most frequently in adults. It is twice as common in females as in males, and its onset is acute in 20%, insidious in 80%. Upper respiratory infection is a definite predisposing factor, and stress is a probable one. Three-fourths of the cases run a chronically progressive course, and one-fourth, an intermittent course, over the first 5 or 10 years. Eventually, almost one-half show appreciable improvement; a few, even apparent recovery.

At least two-thirds of the patients have vasomotor instability, fever, and some leucocytosis at some time or other. In some cases, especially in children with abrupt onset, fever and leucocytosis may be quite high.

Subcutaneous or intracutaneous nodules are found in one-sixth of the cases at a single examination and in more than one-fourth at some time or other. These are relatively large, firm, freely movable, nontender, and often periarticular, and they usually remain for months or years. Appreciable atrophy of the skin of the extremities is seen in some one-third of hospitalized cases. Pigmented areas of skin, trophic changes in nails, erythematous rashes, and nonthrombocytopenic purpura are rare. Psoriasis is seen frequently enough to establish some definite association between it and rheumatoid arthritis, although psoriasis is not regarded as a manifestation of collagen disease per se.

Over one-third of the cases show some (nontender) generalized lymphadenopathy; about one-sixth, some enlargement of the spleen, and a small proportion, some enlargement of the liver.

Although histologic evidence indicates there are some at least minimal cardiac lesions in 50% of cases of rheumatoid arthritis, endocarditis and valvular lesions do not occur and any cardiac sequelae are very rare. Also, chorea does not occur.

Over 20% of the cases have appreciable hypochromic anemia, and 25% of the cases have a low B.M.R. (less than −10%). Rare manifestations include ulcerative colitis, pleuritis, and leucopenia (instead of the commoner leucocytosis).

In the few fatal cases (usually children), glomerulitis is a frequent autopsy finding and amyloidosis is found in some 15%. Terminal pneumonia is the most frequent cause of such deaths.

The only laboratory test which currently shows promise of reasonable diagnostic specificity is the hemagglutination test for rheumatoid arthritis. This con-

sists in demonstrating increased agglutination of sheep cells which have been sensitized by adding small amounts of anti-sheep-cell rabbit serum (and may also include demonstrating decreased inhibition of normal sheep cell agglutinations) by the serum of patients with rheumatoid arthritis.

Separation of juvenile rheumatoid arthritis into a separate category ("Still's disease") does not appear justifiable. The observed differences between adult and juvenile rheumatic arthritis can be explained on the basis of interference with growth plus the tendency of children to manifest systemic symptoms and signs more severely than adults.

It does appear desirable to separate rheumatoid spondylitis ("Marie-Strumpel's disease") into a distinct subclass, made up of cases (predominantly males, in a ratio of about 10:1) with ankylosis of the spine and often the lumbosacral joints, but with peripheral joint involvement at any time in less than 50%, chronic peripheral arthritis in only about 25%, and with other constitutional manifestations limited to some cases with subcutaneous nodules and a few instances of carditis or aortitis; with no elevation of the sedimentation rate in 20%, only moderate elevation in the majority, and with the diagnosis dependent primarily on x-ray findings, criteria for which appear rather variable; it appears uncertain just what proportion of cases in this subclass actually should be considered to have rheumatoid arthritis.

Rheumatic Fever

Rheumatic fever practically always involves the heart. Serial electrocardiograms almost universally show ECG changes. Endocarditis and valvular lesions with permanent sequelae occur with high frequency, as well as myocarditis and, less often, pericarditis.

Of young adults found to have signs of rheumatic heart disease, only 50% have a history of acute rheumatic fever. This indicates that involvement of structures other than the heart reaches clinical proportions in no more than half the cases.

Rheumatic fever in its "typical" form consists of an acute migratory arthritis, usually in children, with high fever, but rarely with leucocytosis with a cell count above 14,000. The larger joints are generally involved, rarely in a symmetrical manner. There is dramatic symptomatic response to salicylates, and the arthritis resolves without permanent damages. Chorea and/or erythema marginatum are frequent. Involvement of the serous membranes may cause abdominal pain. Subcutaneous nodules, also frequent, are usually small, hard, and nontender and tend to disappear after a comparatively short while (weeks).

Multiple reinfection by different strains of Group A streptococci appears essential to set the stage for development of rheumatic fever. About 3% of persons who have a Streptococcus infection develop rheumatic fever some two weeks later. In patients who have rheumatic fever, there are recurrences in from 20% to 50% of those who subsequently develop another Streptococcus infection. All patients with rheumatic fever exhibit antibodies against the commercially available streptolysin-O antigen made by Difco Laboratories, in a titer of at least 100 units/ml.

Lupus Erythematosus

Lupus erythematosus is the most protean of the collagen diseases. The kidney, serous membranes, heart, spleen, and skin are most frequently involved. The

lesions lie in the endothelium of capillaries, arterioles, and venules; in the basement membrane of the glomeruli, and sometimes in extravascular connective tissue.

Kidney involvement is indicated by albuminuria, microscopic hematuria, etc.; renal hypertension is frequent. Splenomegaly and generalized lymphadenopathy are common.

Skin lesions may occur in the form of a diffuse erythema early in the disease, progressing to deep red discoid areas and patchy atrophic scars later, frequently distributed in a butterfly pattern but sometimes occurring elsewhere on the skin or within the mouth. Alopecia is common. Blotchy red macular patterns of the finger tips, dilation of the capillaries around the fingernail beds, and atrophic areas beneath the outer corners of the fingernails are often characteristic.

Fibrinous pleurisy and pleural effusions are among the cardinal manifestations, and complicating bacterial pneumonia is frequent. There is usually an acute transitory polyarthralgia sometime during the course of the disease. An occasional patient will develop chorea, while some develop convulsions or psychoses and a few will have peripheral nerve palsies.

Leucopenia is an outstanding feature unless a secondary infection is superimposed. Most cases show so-called L.E. cells in preparations of blood or marrow. The presence of L.E. cells is almost pathognomonic, but very rarely they are seen with rheumatoid arthritis.

Periarteritis Nodosa

The lesions of periarteritis nodosa are most conspicuous in the walls of small arteries and arterioles. They are widely scattered, exhibit a prominent inflammatory element, and often thrombose. The manifestations of periarteritis nodosa are almost as protean as those of lupus erythematosus.

Low-grade fever with disproportionately elevated white blood cell count is common, and 30% of the cases have eosinophilia. The platelet count is normal, but the bleeding time is often prolonged.

Kidney involvement is major, usually with albuminuria and hematuria, which at times may be gross, and often there is a sudden rise in blood pressure. Fibrinous pleurisy and pleural effusions often are cardinal manifestations. Bronchial asthma may be a serious concomitant or antecedent. Occasionally, a fine nodularity on x-ray resembles miliary tuberculosis or Hodgkin's disease. It is the commonest cause of infarcts of the liver. Polyarthritis is common at some time or other.

Spontaneous subarachnoid hemorrhages and convulsions from local vascular lesions may occur. Peripheral nerve involvement, usually symmetrical, involving isolated nerve trunks or the hands and feet diffusely, or both, is especially characteristic. Muscular involvement is common, and muscle biopsy will frequently show typical histological lesions in the vessel walls. Serous membranes may be involved.

Cutaneous manifestations are seen in one-fourth of the cases and may consist of suddenly appearing, extensive, often symmetrical areas of ecchymosis, which may become necrotic ulcers. Small subcutaneous nodules can often be palpated, but they are not prominent. There may be changes in pigmentation of the skin. Pericarditis and/or myocarditis may occur.

Dermatomyositis

In dermatomyositis the fascial planes of cutaneous tissue and striated muscle, at times also of the myocardium, are most frequently involved. Skin involvement commonly includes more or less all of the subcutaneous and cutaneous tissues. Characteristic lesions are nonpitting, often tender swelling of the subcutaneous tissue and muscles, with marked atrophy in the later stages, and frequently atrophic ulcers and altered pigmentation.

Pulmonary involvement from immobilization of the chest is a frequent cause of death. Leucocytosis is common. There is occasional peripheral nerve involvement and rare kidney involvement.

Scleroderma

Scleroderma is predominantly manifested by diffuse scarring of the superficial dermis and, at times, of widespread areas in the esophagus, intestinal tracts, myocardium, and other organs, including the basement membrane of the glomeruli.

The skin involvement may start in the face, hands, or feet and later involve neck, torso, and shoulders. There is at first diffuse nonpitting edema, then progressive tightening, atrophy, induration, and pigmentary derangement in the skin, with adherence to underlying structures. This may cause marked limitation of the motions of the joints, with eventual trophic ulcers. (When these changes are limited to the fingers, the condition is called sclerodactylia, a localized form of scleroderma which is much commoner than the generalized form and which perhaps should not be classified as a full-blown collagen disease.)

Gastrointestinal lesions are common, the commonest site being in the esophagus, resulting in dysphagia. There is occasionally marked increase in the connective tissue of the myocardium. Rarely, there is involvement of the vessels of the lung and diffuse completely disabling pulmonary fibrosis. Also rarely, the synovia, muscles, and/or kidneys may be involved.

REFERENCES

1. Calkins, E., and Bauer, W.: The Protean Manifestations of the Connective Tissue Diseases, M. Clin. North America 39:325-338, 1955.

2. Hughes, W. F., Jr.: The Ocular Manifestations of Rheumatic Diseases, in Traut, E. F.: Rheumatic Diseases, St. Louis, The C. V. Mosby Company, 1952, pp. 283-300.

3. Johnson, N. J., and Dodd, K.: Juvenile Rheumatoid Arthritis, M. Clin. North America 39:459-487, 1955.

4. Pike, R. M.; Sulkin, S. E., and Coggeshall, H. C.: The Hemagglutination Test for Rheumatoid Arthritis, M. Clin. North America 39:379-391, 1955.

5. Polley, H. F.: The Diagnosis and Treatment of Rheumatoid Spondylitis, M. Clin. North America 39:509-528, 1955.

6. Popper, H., and Lunseth, J. H.: The Collagen Diseases, in Traut, E. F.: Rheumatic Diseases, St. Louis, The C. V. Mosby Company, 1952, pp. 156-178.

7. Rantz L. A.: The Streptococcal Etiology of Rheumatic Fever, M. Clin. North America 39:339-351, 1955.

8. Reynolds, W. E., and Short, C. L.: The Clinical Manifestations of Rheumatoid Arthritis, M. Clin. North America 39:365-377, 1955.

9. Traut, E. F., and Campione, K. M.: Histopathology of Muscle in Rheumatoid Arthritis and Other Diseases, in Traut, E. F.: Rheumatic Diseases, St. Louis, The C. V. Mosby Company, 1952, pp. 144-155.

10. Vail, D.: Diffuse Collagen Diseases with Ocular Complications, Tr. Ophth. Soc. U. Kingdom 72:155-169, 1952.

Evolution of the Collagen Diseases

RENÉ CONTARDO, M.D.
Santiago, Chile

The evolution of the diseases which affect the collagen structures of the eye must be oriented in accordance with the study of the membranes richest in connective tissue—that is, the cornea, which contains more than 90%; the sclera and the iris; the ciliary body; the choroid; the retina, and the optic nerve, which are rich in connective tissue. The vitreous is not included, since it consists of a material similar to collagen.

The diseases of these various membranes are worth studying in connection with generalized diseases, since careful observation of the ocular status in these diseases permits one to see exactly what occurs in other regions of the connective tissue, less accessible to direct examination.

In a large percentage of cases the ocular involvement appears before the general disease becomes evident; hence the discovery of pathological lesions in the collagen structures of the eye, especially when a specific etiologic factor is not encountered, is a warning signal that a detailed general study should be made to avert later involvement of other collagen structures in the rest of the organism.

In a large number of instances ocular lesions are accessible to direct observation, and for this reason they must be taken into account, not only in diseases in which the ocular lesion is evident, as occurs in most cases of rheumatoid arthritis, periarteritis nodosa, and temporal arteritis, but also in diseases in which the development of these lesions is silent, as occurs in dermatomyositis, lupus erythematosus disseminatus, and serum sickness.

Stillerman [74] has noted that collagen lesions not only affect the eye at the same time as they affect the other tissues but also may frequently be observed first only in the eye, presenting the common characteristics of the processes elsewhere.

Von Grolman [78] suggests that there should be a very close collaboration between ophthalmology and the other branches of medicine in order that one may observe the casual factors of diseases which reveal themselves in the eye, since the eye may disclose very early many generalized disorders of mesenchymal structures. He gives the name irritative ocular syndrome to an entity of ocular manifestations which are premonitory and initial symptoms of general disturbances of mesenchymal structures. Vasodilatation and increased capillary permeability in its early stages become apparent in the eye before—at times very long before—a lesion of other organs appears, and, even if the lesions appear simultaneously, the eye offers better conditions for an early diagnosis.

The irritative ocular syndrome denotes a latent disequilibrium of the general health, of mesenchymal structures, or of the reticuloendothelial system. Its

Received for publication March 19, 1956.

National Health Service.

Read in the Symposium on Collagen Diseases at the Fifth Pan-American Congress of Ophthalmology, Santiago, Chile, Jan. 9, 1956.

physiological characteristics are analagous to certain manifestations of the adaptation syndrome of Selye, which includes disorders of various causes with a similar pathogenic mechanism, which set into motion the hypophysial-corticotropic hormone-adrenal cortex mechanism, affecting the various mesenchymal tissues (reticuloendothelial, collagen, muscular, cartilagenous, and bony) and certain elements abundant in these tissues, such as reticulocytes and the lymph nodes.

The irritative ocular syndrome corresponds to the first stages of the irritative disorders, just as the typical disturbances of adaptation constitute the most advanced expressions.

The existence of a collagen disease necessitates a search for possible ocular alterations, while the existance of the irritative ocular syndrome necessitates a search for adaptational disturbances which may exist in the other organs and systems.

Lewis, Sinton, and Knott [50] have noted the fact that one of the characteristic features of the collagen diseases is the multiple involvement of the physical system and the frequency of involvement of the central nervous system in the form of a subclinical mental deficit in certain patients with rheumatoid arthritis.

Recently Polley encountered an association between neoplasms and rheumatism.

Téllez, Corona, Hermosilla, Pelaéz, Yañez, Videla, and Orrego [76] believe that it is probable that in certain collagen diseases there exists a constitutional terrain which predisposes to these diseases and which is based in an alteration of the vegetative centers of the neuroaxis, consisting fundamentally of a reactional disequilibrium of the sympathetic and parasympathetic systems, one of the principal manifestations being the tendency toward sustained angiospasm by reason of the predominance of the adrenergic actions over the cholanergic.

The diverse pathogenic factors act in two ways on this constitutionally predisposed terrain, in one accentuating the aforementioned disequilibrium so that it would be transmitted to the tissues through the intermediation of the neurotropic fibers, or, in the other, affecting it through humoral mechanisms, giving rise to the neurohumoral reactions described by Selye in the so-called adaptation syndrome.

For these reasons, Téllez and his co-workers consider that it is important to make a basic study of the neurotropic mechanisms in the origin of the collagen diseases.

If it is of value to investigate the causes of the collagen diseases, likewise the study of their evolution should be of value.

The collagen diseases may present a process of accelerated aging, similar to that which is produced normally by the stress and strain of daily life.

The general adaptation syndrome of Selye [65] is a complex of reactions to a nocive agent (stress) which starts by a reaction of alarm, with passive phenomena of shock, followed by defensive reactions of countershock, first with the release of epinephrine, which combats hypotension and glycemia, followed by the production of adrenal corticotropic hormone, which stimulates a massive secretion of adrenal corticoids with predominance of the glucocorticoids of the cortisone type, after which there occurs a great increase in lymphocytes, leucocytes with eosinopenia, hyperglycemia, inhibition of scar tissue formation, and so forth. This defense reaction may be prolonged—it is the phase of resistance—but if the harmful agent persists in its nocive action the organism enters the phase of

exhaustion. Selye [65] has shown that under the influence of adrenocorticotropic hormone and of cortisone resistance is increased.

The general syndrome of adaptation in its three characteristic stages has a remarkable resemblance to infancy, adulthood, and senility. In infancy any stimulus produces exaggerated responses, but the infant adapts itself easily to any sort of change in its environment or internal condition. The adult habitually reacts to stimuli in a less violent manner, perhaps because in the course of normal life one acquires a degree of adaptation to many of the stimuli, but as age increases, the capacity of adaptation diminishes until it reaches its lowest point in extreme old age.

The general characteristics of the disorders of adaptation coincide with the so-called degenerative diseases of age.

In the diseases of adaptation the part sensitive to the hormonal disequilibrium is the collagen tissue, as it also is to certain factors in the phenomena of diffusion and to some of the enzymes. Collagen tissue is a tissue permeable to water, electrolytes, and products of metabolism, hyaluronic acid being one of the principal maintainers of the viscosity and the degree of impermeability of the tissues, thus regulating the phenomena of diffusion. Exogenous and endogenous enzymes affect the collagen tissue, which it is very sensitive to them, as it is to one of the best-known factors of diffusion, hyaluronidase, which hydrolyzes and polymerizes hyaluronic acid and increases the permeability of the connective tissue. Under its influence there will occur fibrinoid change which may progress to sclerosis of the collagen fibers.

Cortisone and adrenocorticotropic hormone are powerful inhibitors of the enzymatic effect of hyaluronidase, as has been demonstrated experimentally. If a rabbit is injected with trypan blue, its skin does not change color, but if hyaluronidase is added to the trypan blue its skin turns blue, but this phenomenon does not occur if cortisone has been injected before the dye is injected.

Cortisone produces very good results in inflammation of the mesenchymal tissues, with which the eye is very well supplied. It exerts an anti-inflammatory action on the ocular tissues, and its most favorable results occur when there is more exudative activity, vascular ingurgitation, and formation of new vessels. Marin-Amat [52] believes that cortisone locally, since it is antiphlogistic, acts upon all and each of the factors which bring about inflammation, for which reason it acts upon the sympathetic system, diminishing the vascular lumen, decreasing or abolishing the permeability of the endothelium and lymphatic extravasation. Its activity is nonspecific; it has no effect on the causes producing the pathological alterations. Hobbs [37] believes that its effect is due to a blocking of the exudative reaction of the uveal tract irritated by an abnormal stimulus. Fritz [33] interprets it as a block of the action of the sympathetic nerves on the blood capillaries. Cortisone locally produces a decrease and a stabilization of the pressure of the blood capillaries of the eye, at the same time reducing the quantity of blood in the capillaries, an effect which is of great value since in the inflammatory conditions of the eye capillary pressure is increased.

As cortisone and adrenocorticotropic hormone are so effective in collagen diseases, the effects of treatment with them and the progress and remission have been much better understood because the internal ocular structures may be seen and studied during treatment. Although ocular lesions are often absent in many cases of dermatomyositis, lupus erythematosus disseminatus, serum sickness,

and periarteritis nodosa, it is necessary to make a complete ocular examination, including ophthalmoscopy, when a collagen disease is suspected, and if the diagnosis is confirmed the ocular examination should be repeated frequently.

I have assembled 200 cases of diseases of the cornea, sclera, ciliary body, choroid, retina, and optic nerve, and in studying their evolution I have encountered the following conditions:

1. Cornea
Recurrent corneal ulcers
Keratoconjunctivitis sicca, or Sjögren syndrome
Superficial or deep keratitis
2. Sclera
Recurrent scleritis
Sclerouveitis
3. Uvea
Midriasis
Iritis
Iridocyclitis
Choroiditis
Uveitis
4. Retina
Retinopathy
Obstruction of the central artery
5. Optic nerve
Capillary hyperemia and dilated and obscured veins
Capillary edema

Of the diseases of the eye, keratitis may appear in band form, disciform, or sicca; the last, whose exact name is keratoconjunctivitis sicca, constitutes the clinical basis of the syndrome of Sjögren, which presents, in addition, dryness of the mouth and throat and swelling of the parotid glands, produced by a chronic inflammatory atrophy of the salivary and lacrimal glands. The qualitative and quantitative secondary deficiency of tear production reaches the point of epithelial exfoliation and interferes with the regeneration of the corneal epithelium.

The association of keratoconjunctivitis sicca and rheumatoid arthritis was first mentioned by Houwer,[40] in 1927; in 1933 Sjögren [68] published an original study of 22 cases of which 17 presented rheumatoid arthritis. In 1948 Espildora Luque, Mosser, and Gormaz,[29] in 9 cases of the Sjögren syndrome, in all of which the patients had suffered or were suffering from rheumatoid arthritis, and Holm and Stenstam [39] in 1948-1949, in a clinical material of 500 patients with rheumatoid arthritis, encountered the frequency of 14.2% of keratoconjunctivitis sicca.

Lackington, Charlin, and Gormaz,[47] in 50 cases of rheumatoid arthritis encountered an incidence of 34% of keratoconjunctivitis sicca; of these patients, 46 were women. They observed that the onset of the keratoconjunctival picture is generally later by several years than the appearance of the articular picture and that there is a certain correlation between the intensity of ocular process and the severity of the rheumatoid arthritis. Appelmans, Michiels, and van Assche [2] do not wholly agree, in spite of the fact that they believe that the two conditions progress and regress together.

Scleral involvement in collagen diseases is frequent, notwithstanding that scleral diseases are relatively rare, but they must not be considered as purely local as Stillerman,[74] Swan,[75] and Klemperer [45] have suggested.

Radnot [61] reports a case of a woman of 43 years who was suffering from articular rheumatism and chronic uveitis in which a progressive posterior scleritis

appeared, and Babel [9] reported the case of a patient, a man, with acute articular rheumatism in which a scleritis and acute episcleritis appeared; this extended to the extraocular muscles, to the surrounding orbital tissue, and to the extraocular membranes, producing a retinal detachment.

Goar and Smith [35] report two cases of perilimbal rheumatoid nodules in a man of 65 years and a woman of 48 years as manifestation of fibrinoid degeneration in a collagen disease, a disease which has been called scleromalacia perforans, since the scleral nodule progresses by way of secondary infection to ulceration and necrosis. On histopathologic examination in the first case the cornea and the sclera were partially necrotic and infiltrated with purulent exudate with the presence of lymphocytes, plasma cells, and polymorphonuclear cells in the uveal tract. In the second case several years later the other eye became involved, with loss of vision. Histopathologic examination showed scleral thickening of 6 mm. with foci of necrosis, surrounded in parts with epithelioid cells, lymphocytes, and macrophage pigment. The cornea was opaque; the retina detached, with a hemorrhage behind the choroid and perforation of the sclera, with collapse of the globe. From the histologic point of view the scleral lesions showed characteristics similar to those which may be seen in the articular rheumatoid nodule.

It is important to recognize these lesions in their early form, since the ocular signs may appear before the articular signs, an association which, permitting an etiological diagnosis, makes possible correct management with adrenal cortex therapy, in spite of a case of Anderson and Margolis in which treatment with cortisone and corticotropin was unavailing. This was a case of rheumatoid arthritis which had been developing for 10 years which led to blindness in one eye and to light perception in the other with exophthalmos, hypotony, scleral and corneal anesthesia, posterior synechias, cataract, and precipitates in the membrane of Descemet, with necrosis of the anterior segment to the equator and with formation of cavities which developed almost to the point of exposing the exterior uvea, which was covered by a thin opalescent lamina, but there were no inflammatory signs—a picture which led to bilateral enucleation in spite of the treatment with cortisone and corticotropin. The histopathologic study showed necrobiotic nodules of rheumatoid arthritis characterized by multiple foci of fibrinoid degeneration and necrosis surrounded by palisades of fibroblasts and epithelioid cells which completely destroyed the scleral layers. Around the scleral lesion were infiltrates of plasma cells, particularly the perivascular regions as well as the sclera, the episclera, and the choroid.

Ashton and Hobbs believe that scleromalacia perforans ought to be included among the collagen diseases on the basis of clinical and anatopathological findings, and for this reason they prefer the term rheumatoid nodules of the sclera. They describe a case treated with local and general cortisone with which they proved clinically leveling off and retraction of the nodules, with acceleration of cicatrization, but, in spite of the fact that the lesions became covered with a newformed fibrous tissue, no complete cure. The death of the patient of septic pericarditis while the lesions were in process of evolution permitted histopathologic study of one of the eyes; in this they found a marked inhibition of inflammatory infiltration, particularly in respect to the polymorphonuclear neutrophiles and eosinophiles, as well as a marked and definite fibroblastic regeneration associated with a uniformly oriented orderly arrangement of elements

around the zones of fibrinoid necrosis. They arrived at the conclusion that cortisone prevents the final activity, creating favorable conditions for fibrous formation of fibrous tissue, but at the same time the concentration of the hormone proportionately inhibits the regenerative process, a condition which must be taken into account in determining the dosage.

I observed the case of a diabetic woman of 63 years who presented a 2 by 1 cm. fusiform scleral tumoral mass, whitish and hard, in the superior internal quadrant of the globe coinciding with an old iritis. Except for diabetes, the rest of the tests of all sorts which were made were negative. She was treated with all the therapeutic means known, including radiotherapy, without result. The condition progressed to secondary glaucoma, which, because of the intense pains, led to enucleation. The histopathologic examination revealed a fusiform thickening of the sclera, which measured 8.5 mm. in its widest part, formed of fibrous tissue with areolas and plasmocellular infiltrates; both the sclera and the uvea presented intense diffuse inflammatory infiltrates composed of lymphocytes and a great quantity of plasma cells. Altogether, it was a matter of hyperplastic sclerouveitis. Later the other eye became blind because of iridocyclitis in spite of treatment with cortisone (all due precautions being observed because of the diabetes), a drug which during its use greatly improved the condition both subjectively and objectively, but as soon as its use was stopped the vision fell to light perception and new fine whitish precipitates appeared in the membrane of Descemet.

Iritis and iridocyclitis when they appear in connection with collagen diseases constitute a peculiar response of the iris and the ciliary body, due to their connective tissue structure, a fact which is of great interest in view of the fact that one of the most difficult problems of ophthalmology is the determination of the etiology of the diseases of the uveal tract.

According to Zwiauer, Deutsch, and Streit,[80] iritis and iridocyclitis represent the localization of a general or focal disease, creating a propitious terrain for the development of a pathologic affection; for this reason, they believe that not only the eye but also the rest of the organism should be affected, one of the first symptoms of a pathologic ocular affection being the change of permeability of the blood-tissue barrier which causes a greater passage of albumin into the tissues and with it morphologic changes in the iris and ciliary body. These authors have shown that when iritis and iridocyclitis appear there exists a total disturbance of the permeability of the organism and an alteration of the albuminous aspect.

The characteristic of the corneal, scleral, and uveal lesions has been their reappearance and their resistance to any sort of treatment even after every possible etiologic cause has been eliminated. Moreover, in certain cases the ocular involvement appeared before the development of the general disease, a fact which makes an important point in interpreting the collagen diseases. Thus in 10 cases of rheumatoid arthritis which we observed, 1 of which had developed over a 12-year period, it was possible to determine the positivity of the Schirmer test before articular lesions existed. In a recent case, that of a woman aged 44 years with a clinical diagnosis of neurovegatative dystonia with a predominance of the adrenergic tone, the Schirmer test was positive, a fact which lends support to the idea of an existence of a disequilibrium of the sympathetic system in collagen disease.

In a series of 50 cases treated between 1947 and 1955 in which we were not able to determine the etiology, it was possible to observe the repetition of various pictures in distinct epochs, affecting one or the other of the ocular membranes rich in connective tissue, in the form of a recurrence of the previous affection or even involving others of the collagen structures of the eye.

This fact is of great importance, since the establishment of involvement of the collagen structures of the eye implies the possibility of repetition of the same picture in a later stage or others of the collagenous membranes of the eye, besides that it is a sign to suspect some general collagen disease in the future, since in a great percentage of cases that is the manner of development of many of the ocular collagen diseases.

The evolution of the diseases of the cornea, sclera, uvea, and optic nerve ought to be studied in ways that make possible the determination of a possible etiologic cause, and if this cannot be done then they must be treated to prevent the involvement of the rest of the collagen structures of the organism. In this therapy the use of cortisone, corticotropin, prednisone, and so forth, is of great help; they influence favorably the course of those affections since they have a great effect on the connective tissue and permit striking improvement in the prognosis of the diseases of the collagen structure of the eye and of the rest of the body.

It is interesting to note the observed relation of the collagen diseases and glaucoma. In five cases of rheumatoid arthritis there was also glaucoma, which certainly may have been a mere coincidence, since glaucoma is a disease of old age which increases after the age of 40 years, given the fact that with the years the arterial sclerotic processes acquire greater intensity and are of more importance in the physiology of the intraocular circulation. The patients varied in age between 48 and 60 years, a fact which may explain that association.

Stillerman [74] believes that it is possible that open angle glaucoma may be due to an alteration of the connective tissue, a hypothesis which it is necessary to consider given the fact that the drainage system of the anterior chamber is situated in the sclera, formed by collagenous fibers in its totality; likewise the scleralcorneal trabecula, the internal canals of Sonderman, the canal of Schlemm, the external collector canals, the deep intrascleral plexus, and the aqueous veins, by reason of their situation, are subject to the alterations peculiar to the connective tissue.

But our five cases, two of acute form and three of chronic form, were gonioscopically proved to be narrow-angle cases, in which, in our opinion, hypertension has a mechanical origin by blockage of the angle at the base of the iris. Nevertheless, it should be called to attention that in narrow-angle glaucoma vascular congestion dominates and in the collagen diseases that which dominates the pathologic alterations is their eminently vascular character, as pathologic anatomy has shown; this reveals that these affections compromise the small arteries and the capillaries by a lesion of the connective tissue of their walls.

REFERENCES

1. Anderson, B., and Margolis, G.: Am. J. Ophth. 35:917, 1952.
2. Appelmans, M.; Michiels, J., and van Assche, E.: Ophthalmologica 122:337, 1951.
3. Armas Cruz, R., and Harnecker, J. J.: Rev. méd. Chile, Supp. 1, p. 1, 1950.
4. Armas Cruz, R.; Harnecker, J., and Parrochia, E.: Rev. méd. Chile 80:449, 1952.
5. Ashton, N., and Hobbs, H. E.: Brit. J. Ophth. 36:373, 1952.

6. Baehr, G.; Klemperer, O., and Schifrin, A.: Tr. A. Am. Physicians 50:139, 1935.
7. Bauer, W., and Kulka, J. P., read before the Seventh International Congress on Rheumatic Diseases, New York, May 30-June 3, 1949.
8. Berardinis, E., and Bonavolonta, G.: Arch. ottal. 55:487, 1951.
9. Babel, J.: Ann. ocul. 179:540, 1946.
10. Berliner, M. L.: A. M. A. Arch. Ophth. 47:250, 1952.
11. Burk, M.: Klin. Monatsbl. Augenh. 120:273, 1952.
12. Cadman, E. F. B., and Robertson, A. J.: Brit. M. J. 2:68, 1952.
13. Cecil, R.: A Text-Book of Medicine, Philadelphia, W. B. Saunders Company, 1944.
14. Charlin Correa, C.; read before the International Congress of Ophthalmology, Washington, D. C., April 25-28, 1922.
15. Charlin Correa, C.: Ann. ocul. 158:861, 1921.
16. Coburn, A. F., and Moore, D. H.: Bull. Johns Hopkins Hosp. 73:196, 1943.
17. Contardo, R.: Curso de oftalmologia, Escuela de Medicina, Universidad de Chile, Editorial Universitaria, 1953.
18. Contardo, R.: Esclerouveitis hiperplastica, read before the Ophthalmological Society of Chile, Oct. 3, 1953.
19. Contardo, R.: Enfermedades del colágeno, Arch. brasil. oftal. 17:114, 1954.
20. Contardo, R.: Fundamentos gonioscópicos en la pathogenia y circugía del glaucoma primitivo, Thesis, Santiago, Chile, 1955.
21. Costa, C.: Arteritis y flebitis de los vasos temporales, unpublished data, 1948.
22. Ducach, G., and Cranier, J., read before the Medical Society of Santiago, Sept. 15, 1950.
23. Duff, G. L.: Canad. M. A. J. 58:317, 1948.
24. Duke-Elder, W.: Text-Book of Ophthalmology, St. Louis, The C. V. Mosby Company, 1941.
25. Duke-Elder, S.: Brit. J. Ophth. 35:637, 1951.
26. Ellman, P.; Weber, F. P., and Goodier, T. E. W.: Quart. J. Med. 20:33, 1951.
27. Espildora Luque, C.: Etiologia del glaucoma primitivo, Thesis, School of Medicine, University of Chile, 1921.
28. Espildora Luque, C., read before the International Congress of Ophthalmology, Washington, D. C., April 25-28, 1922.
29. Espildora Luque, C.; Mosser, J., and Gormaz, A.: Arch. Soc. oftal. hispano-am. 8:333, 1948.
30. Espildora Luque, C., and Coutts, W.: Ojo y lifogranuloma venéreo, Santiago, Chile, Ed. Zig-Zag, 1947.
31. Fitzgerald, J. R.; Bellows, J. G.; Donegan, J. M.; Gamble, R. C.; Krause, A. C.; Mann, W. A.; Pearlman, M. D., and Zekman, T. N.: A.M.A. Arch. Ophth. 45:320, 1951.
32. Frenkel, M.; Hellinga, G., and Groen, J.: Acta endocrinol. 6:161, 1951.
33. Fritz, M., in discussion on Calamandrei, G., and Ferrata, L.: Bull. et mém. Soc. franç. opht., p. 468, 1951, p. 487.
34. Garreton Silva, A.: El Sindrome general de los glaucomatosos, Thesis, School of Medicine, University of Chile, 1923.
35. Goar, E. L., and Smith, L. S.: J.A.M.A. 148:889, 1952.
36. Hargraves, M. M.; Richmond, H., and Morton, R.: Proc. Staff Meet. Mayo Clin. 23:25, 1948.
37. Hobbs, H. E.: Tr. Ophth. Soc. U. Kingdom 71:475, 1951.
38. Hollenhorst, R. W., and Henderson, J. W.: Am. J. M. Sc. 221:211, 1951.
39. Holm, S., and Stenstam, T.: Acta med. scandinav. 127:130, 1947.
40. Houwer, A. W. M.: Tr. Ophth. Soc. U. Kingdom 47:88, 1927.
41. Keith, N. M., and Rowntree, L. G.: Tr. Am. A. Physicians 37:487, 1922.
42. Klemperer, P.: Bull. New York Acad. Med. 23:581, 1947.
43. Klemperer, P.; Pollack, A. D., and Baehr, G.: J.A.M.A. 119:331, 1942.
44. Klemperer, P., read before the Seventh International Congress of Rheumatic Diseases, New York, May 30-June 3, 1949.
45. Klemperer, P.: Am. J. Path. 26:505, 1950.
46. Kratka, W. J.: Am. J. Ophth. 36:510, 1953.
47. Lackington, M. C.; Charlin, V. C., and Gormaz, B. A.: Rev. méd. Chile 79:233, 1951.
48. Lepri, G., and Ergoli, G.: Rass. fisiopat. clin. 24:617, 1952.

49. Lepri, G.: Ann. ocul. 186:351, 1953.
50. Lewis, B. I.; Sinton, D. W., and Knott, J. R.: A.M.A. Arch. Int. Med. 93:315, 1954.
51. Maumenee, A. E.: Am. J. Ophth. 23:971, 1940.
52. Marin-Amat, M.: Arch. Soc. oftal. hispano-am. 13:121, 1953.
53. Maximow, A. A., and Bloom, W.: Tratado de histologia, Buenos Aires, Ed. Labor., 1947.
54. Magitot and Baillart: Ann. ocul. 163:937, 1926.
55. Navarro Patiño, A.: Arch. Soc. oftal. hispano-am. 12:1064, 1952.
56. Offret, G., and Forest: Bull. Soc. opht. France, p. 759.
57. Pasqualini, R. Q.: Stress, Buenos Aires, El Ateneo, 1952.
58. Philips, C.: A.M.A. Arch. Ophth. 48:518, 1952.
59. Pillat, A.: von Graefes Arch. Ophth. 133:566, 1935.
60. Piñero y Carrion, A.: Arch. Soc. oftal. hispano-am. 12:300, 1952.
61. Radnot, M.: Ophthalmologica 116:167, 1948.
62. Rapaport, M.: Arch. Soc. argent. anat. 9:67, 1947.
63. Rinchart, J. R., read before the Seventh International Congress on Rheumatic Diseases, New York, May 30-June 3, 1949.
64. Rodriguez, L.: Glaucoma y colesterol, Thesis, University of Chile, 1953.
65. Selye, H.: The Physiology and Pathology of Exposure to Stress, Montreal, Acta, Inc., 1950.
66. Selye, H.: J. Clin. Endocrinol. 6:117, 1946.
67. Silva, C.: Enfermedades colágenas, Bol. hosp. Viña del Mar 7:95, 1951.
68. Sjögren, H.: Acta ophth., Supp. 2, p. 1, 1933.
69. Scharz, cited by Thiel, 1953.
70. Middleton, W. S.: M. Clin. North America 37:1697, 1953.
71. Stephens, C. A. L.: in Proceedings of the First Clinical ACTH Conference, edited by J. R. Mote, Philadelphia, The Blakiston Company, 1950, p. 358.
72. Steffensen, E. H.; Ivy, H. B., and Nagle, F. O.: Am. J. Ophth. 35:933, 1952.
73. Stewart, L. L.: J. Michigan M. Soc. 48:344, 1949.
74. Stillerman, M. L.: A.M.A. Arch. Ophth. 45:239, 1951.
75. Swan, K. C.: A.M.A. Arch. Ophth. 45:630, 1951.
76. Téllez, R.; Corona, L.; Hermosilla, M.; Pelaez, P.; Yañez, A.; Videla, P., and Orrego, A.: Rev. méd. Chile 79:458, 1951.
77. Thiel, R., read before the Fifth Argentine Congress of Ophthalmology, Buenos Aires, April 15, 1953.
78. von Grolman, G., read before the Fourth Pan-American Congress of Ophthalmology, Mexico, D. F., Jan. 6-12, 1952.
79. Zañartu, J., and Medina, E.: Rev. méd. Chile 79:4, 1951.
80. Zwiauer, A.; Deutsch, E., and Streit, M.: Klin. Monatsbl. Augenh. 122:655, 1953.

The Therapy of Collagen Diseases

IRVING H. LEOPOLD, M.D.
Philadelphia

Nature of Collagen Diseases

Collagen diseases may be characterized as a group of disorders of unknown etiology that have in common histologic alterations of the connective tissues. They are of particular interest to the ophthalmologist because they frequently produce ocular changes. A typical connective tissue lesion in these diseases is fibrinoid degeneration. This common histopathologic change does not indicate a common etiology. Connective tissue may respond to a large variety of traumatic agents with only a few nonspecific reactions; thus various stimuli can produce the same changes in connective tissue. The reactions demonstrated by connective tissue are necrosis, fibrinoid alteration, cellular infiltration, sclerosis, proliferation, or various combinations of these.

Klemperer has defined these collagen diseases as clinically unrelated conditions which are similar only in that most of them appear to be due to a hypersensitivity reaction and all have a peculiar fibrinoid degeneration of connective tissue. It is thought that the fibrinoid tissue response represents an altered or precipitated protein-mucopolysaccharide substance which is laid down by altered connective tissue cells between the collagen fibers. Experimentally, fibrinoid degeneration has been produced by the direct action of toxins, by the use of enzymes such as hyaluronidase or collagenase, after deficient diets, and in experimental hypertension (Stillerman).

Collagen Diseases

The diseases which are usually included in this group are disseminated lupus erythematosus, periarteritis nodosa, scleroderma, dermatomyositis, rheumatoid arthritis, temporal arteritis, and rheumatic fever. Other diseases have been added, such as thromboangiitis obliterans, erythema nodosa and erythema multiforme, anaphylactoid purpura, antigenic pneumonitis, serum sickness, and, probably, calcinosis. As time passes others may be added and some of the diseases now considered members may be removed from this group.

In addition to the presence of fibrinoid degeneration of the connective tissue, other common factors in collagen diseases are fever, increased sedimentation rate, and, usually, joint pain and swelling. Not all of these signs are present in every one of this group; for example, serum sickness is not usually accompanied by an increased sedimentation rate. Sometimes one may find calcium retention, as in scleroderma, dermatomyositis, and calcinosis.

Ocular Manifestations

The eye is involved in these disease processes because the eye contains collagenous fibers. Such fibers are found in the lids, cornea, subepithelial tissue of

Received for publication March 4, 1956.

Graduate School of Medicine, University of Pennsylvania and Wills Eye Hospital.

Read in the Symposium on Collagen Diseases at the Fifth Pan-American Congress of Ophthalmology, Santiago, Chile, Jan. 9, 1956.

the conjunctival and the episcleral tissue, and in the meshwork of the angle, in the stroma of the iris, ciliary body, and choroid (Barany believes that the trabeculum is made up of a central core of collagen surrounded by elastic material and this is surrounded in part by hyaluronic acid), sclera, Tenon's capsule, the tendons of the extraocular muscles, within the walls of all the blood vessels, and in the supportive structure of the optic nerve and its sheath. Other structures, such as the retina and vitreous, may be involved secondarily as a result of changes in the retinal vessel walls (Table 1).

TABLE 1.—*Ocular Manifestations of Collagen Disease*

Lids	Erythema; edema	
Conjunctiva	Phlyctenules, hyperemia, edema	
Cornea	Sclerosing keratitis, band	
Sclera	Episcleritis, nodular and brawny scleritis, scleromalacia perforans	
Uvea	Uveitis, choroidal infarcts	
Retina	Hemorrhage (petechial, superficial) Exudates, cytoid bodies Vascular Periphlebitis Periarteritis Occlusions	
Visual fields	Vascular lesions in optic pathways	
Pupil	Dysfunction	Vascular lesions in central nervous system
Extraocular muscle	Palsies	Vascular lesions in central nervous system
Glaucoma		
Optic nerve	Neuritis	

Connective Tissue Alterations

The therapy of this type of disease process, since we are not certain of any common etiology, necessitates finding some measures for blocking the reactions in the connective tissues which lead to observable abnormalities. It is helpful, therefore, to know something of the chemical and histologic structure, the metabolism, and the responsive nature to stimuli of this particular type of tissue.

The connective tissue is composed of cellular, chiefly fibroblast, and fibrillar (collagen, elastin, and reticulin) elements held together by a third element, the amorphous ground or cement substance. The latter is a colloid of unknown origin (Stillerman). This ground substance contains hyaluronic acid, a mucopolysaccharide, which is the substrate on which spreading factors, such as hyaluronidase, and other mucolytic enzymes act. Various substances, in addition to enzymes, will affect the permeability of the ground substance. Hormones, such as cortisone, corticotropin (ACTH), and gonadotropins, will produce these changes. It is known that various infecting agents which possess the enzyme hyaluronidase can spread through the connective tissue much more readily than organisms which do not possess this enzyme.

Collagen fibers are wavy branching threads of 1μ to 12μ in thickness, with longitudinal striation. Reticular fibers are considered by some to be the precursors of the collagen fibers. Collagen is a simple protein which can be broken down into certain amino acids, depending on the particular species from which it has been removed. Collagen can be digested by certain enzymes such as pepsin but does resist an enzyme like trypsin. Collagen can be changed to gelatin by boiling.

Kornblueth, working with ocular tissue, has confirmed Klemperer's original finding that the fibrinoid tissue reaction is due to an altered or precipitated protein-mucopolysaccharide material elaborated by malfunctioning connective tissue cells lying between the collagen fibers. Some of these fibrinoid changes are reversible.

Whether or not fibrinoid degeneration is present, another reaction, which is common to most of these diseases, is fibroblastic proliferation. The new fibroblast invades any necrotic mass which may be present and lays down new collagen fibers. This new fibrous connective tissue can itself give rise to fibrinoid necrosis. Thus the fundamental pathologic process in the connective tissue consists of two aspects, destruction and proliferation. Different tissues may show a different degree of response in these two pathologic processes. Likewise there may be a different response in the degree of inflammatory reactions which are associated with fibrinoid necrosis and proliferation. These differences together with the differences in anatomical distribution of the lesions permit the recognition of distinct disease entities on pathological as well as clinical grounds (Vail).

Therapy

Nonspecific therapy in these conditions can be directed toward preventing the development of fibrinoid reactions or inducing their reversal when they occur. In addition, one can take measures to prevent the proliferation of fibroblastic tissue and to reduce inflammatory reaction. If one knew the specific enzymes involved, he could use enzyme inhibitors if such agents were available. Trigentisic acid is thought to be an inhibitor of hyaluronidase.

Occasionally one may be specific in collagen disease therapy. For example, periarteritis nodosa at times has been demonstrated to be due to hypersensitivity to the use of sulfonamides. Cessation of sulfonamide therapy may bring about (Reinhardt and Waldron). Cessation of therapy with this drug has also brought arrest of the disorder. Lupus erythematosus has been reported as occurring after the use of hydralazine (Apresoline) for the treatment of hypertension about a remission of the disease induced by its use.

Therapy of collagen diseases of the eye may be divided into the following groups: (1) specific measures against etiologic factors, (2) measures directed against connective tissue changes, and (3) measures for relief of the patient's discomfort.

Symptomatic therapy may be systemically and locally administered. The general health of the patient must be maintained at an optimal level. Analgesics and sedatives are certainly of value for aiding the patient's morale. Measures to reduce the local reaction consist of anti-inflammatory agents such as the steroids, antihistaminics, and autonomic drugs, e. g., atropine and epinephrine (Table 2).

Specific Therapy

In addition to the removal of allergens and specific desensitization in the therapy of collagen disorders, it should be noted that there are types of collagen diseases which seem to be associated with neoplastic disease (Lansbury). The relationship between malignancy and dyscollagenoses is not coincidental, since connective tissue reaction may disappear following resection of a cancer. The connective tissue manifestations may precede the localized signs of the malignancy itself.

TABLE 2. *Therapy of Ocular Manifestations of Collagen Diseases*

Specific Therapy ⟶		1. Removal of allergens Bacterial Viral Drug 2. Desensitization 3. Removal of neoplasm?
	Systemic	Local
Nonspecific therapy ⟶	1. Steroids Prednisone Prednisolone	1. Physiotherapy Cold Heat Diathermy Irradiation
	2. Phenylbutazone	2. Cycloplegics
	3. Salicylates	3. Antihistaminics
	4. Gold salts	4. Steroids as under "Systemic"+fludrocortisone
	5. Potassium salt of *p*-aminobenzoic acid	
	6. Anticoagulants	
	7. Vasodilators	5. Vasodilators
	8. Transfusions	
	9. Irradiation	6. Stellate ganglion block
	10. Quinacrine	7. Retrobulbar injections
	11. Chloroquine	
	12. Daraprim	8. Surgical measures Tarsorrhaphy Paracentesis Antiglaucoma therapy, etc.
	13. Mechlorethamine Triethylene melamine	

There is a syndrome known as the Bamberger-Marie syndrome which is characterized by new periosteal growth of long bones, increased size of extremities, clubbing of fingers and toes, and redundancy of the skin of the face and skull. The joints may be swollen and deformed. It may look very much like rheumatoid arthritis. There may be peripheral neuritis and phlebothrombosis of the extremities or of the eyes, which may be associated with a pulmonary malignancy. Association of dermatomysitis with malignancy was first reported by Bezecny in 1935. Lansbury has added six cases of collagen disease, dermatomyositis, disseminated lupus erythematosus, and arthritis, complicating carcinoma of the kidney, carcinoma of the esophagus, and Kruckenberg tumor of the colon. The mechanism by which malignancy and connective tissue disorders are related is unknown. Current speculations include infection, allergy, hormones, metabolism, and neurologic reflexes. Nevertheless, the occurrence of this type of syndrome or any of the collagen diseases in a middle-aged patient calls for meticulous clinical, radiographic, and endoscopic study for detection of early operable malignancy.

Nonspecific Therapy

Many drugs have been used for the therapy of collagen disorders, such as quinacrine (Atabrine), daraprim, and chloroquine (Aralen) (Kierland; Dubois; Tye). There are several reports in the literature that quinacrine therapy is of help in lupus erythematosus. Usually the dose is 100 mg. of quinacrine hydrochloride three times daily the first week, twice daily the second week, and then once daily. Significant improvement appears within the first four to six weeks in those who are to be benefited by such treatment. In patients with the systemic variety of lupus, the drug is hazardous as well as unsatisfactory. The untoward reactions with this type of therapy are the yellow color of the skin, which dis-

appears a few weeks or months after discontinuance of the drug and fluorescence of the nails, teeth, and sweat ducts, which takes longer to disappear after the agent is discontinued; occasionally gastrointestinal reactions are noted and sometimes anxiety and increased nervousness. Dubois feels that quinacrine is definitely effective in the treatment of discoid, chronic systemic, and subacute systemic lupus.

Daraprim in a dose of 25 mg. a week then raised in two steps to 25 mg. daily produced only slight improvement in the few patients treated.

Chlorquine phosphate may be given as 0.25 gm. four times daily for two days, 0.25 gm. three times daily for two days, and then 0.25 gm. twice a day. It has been reported by Tye and his group to bring about considerable improvement in patients with chronic discoid lupus erythematosus.

Sterile mechlorethamine (Mustargen) has been given in lupus as a single dose of 20 mg. injected into the tubing of an intravenous infusion of 5% dextrose in water. For similar therapy investigators have ordered triethylene melamine (TEM) taken in water on an empty stomach one hour before breakfast. TEM has been given every two or three days until a total dose of 10 to 15 mg. was administered. All the patients who were given this therapy by Dubois had been previously taking cortisone or corticotropin, and this medication was continued throughout. They had not been benefited by the former steroid therapy. The best results were obtained in those patients who were edematous with the nephrotic type of nephropathy.

Corticotropin and hydrocortisone, cortisone, prednisone, and prednisolone have received the most attention for the treatment of these conditions. Usually large initial doses are used, and corticotropin is most frequently employed for initial therapy. Corticotropin is usually given as 100 mg. intramuscularly in four doses, or, in the more acutely ill patients, 20 to 40 mg. is given intravenously dissolved in 500 to 1000 cc. 5% dextrose and given slowly over eight hours. When cortisone was used initially, daily amounts varied from 200 to 800 mg. orally in four divided doses. Hydrocortisone may be used in similar concentrations, and prednisone or prednisolone, in amounts varying from 30 to 100 mg. daily. The average daily maintenance dose after the disease has been brought under control is between 50 and 100 mg. of cortisone, between 25 and 75 mg. of corticotropin, or between 15 and 40 mg. of prednisone or prednisolone (Soffer; Harvey).

In the systemic therapy of ocular manifestations of rheumatoid arthritis attention has been paid not only to cortisone and corticotropin but to salicylates and combinations of salicylates with steroid agents. There is no doubt that many of the systemic symptoms of this disease can be controlled with the constant use of steroids. However, there is evidence that the joint signs may progress although the symptoms have been eliminated. On the other hand, the British investigators (Joint Committee of Medical Research Council) have shown that salicylates may be sometimes as helpful as steroids, and more recently it has been demonstrated that the combination of salicylates with steroids may allow each of them to be used in smaller doses and produce a heightened effect (Spies). Unfortunately, not all cases of ocular manifestations associated with rheumatoid arthritis benefit from such therapy. (I have seen a case of scleromalacia proliferans develop during active corticotropin and steroid therapy which failed to subside in the presence of intensive local and systemic use of steroids, phenylbutazone (Butazolidin), salicylates, and other measures. According to the Joint Committee of the Medical Research Council and the Nuffield Foundation, it was felt that there was little

to choose between the cortisone-treated group and the patients maintained on acetylsalicylic acid in the early stages of rheumatoid arthritis. The mean maintenance dose to bring about equal improvement was 80 mg. cortisone after a year of therapy and 4.5 gm. daily of acetylsalicylic acid.

Smith and his co-workers attempted to measure the adrenocortical steroids in the urine of patients receiving large doses of salicylates in order to find out whether salicylates were causing stimulation of the pituitary or the adrenal cortex or both. Their results would indicate that, although there are some similarities in metabolic and clinical effects of salicylates and of corticotropin and cortisone, the differences are such that the theory that therapeutic activity of salicylates in rheumatic diseases depends on intermediary production of corticotropin is untenable. Done and his co-workers were able to show an elevated 17-hydroxycorticosteroid concentration in patients with salicylate intoxication and in guinea pigs given large doses of salicylates, but they could not determine whether the increased concentration of steroid reflected a specific effect on the pituitary-adrenal system, nonspecific stress response, or an alteration in mechanisms concerned with the removal of 17-hydroxycorticosteroids from the circulation. Bayliss and Steinbeck did not find increased plasma levels of 17-hydroxycorticosteroid either in patients receiving prolonged treatment with acetylsalicylic acid or in those given large doses of sodium salicylates sufficient to raise the plasma level to 20 mg/100 cc. Thus it is not apparent how salicylates bring about their action. It is not clear-cut that they act through the pituitary-adrenal axis by stimulating these particular organs directly.

There is also some evidence that the addition of *p*-aminobenzoic acid along with cortisone or along with salicylates might improve the results in rheumatoid arthritis (Zarafonetis) (Wiesel and Barritt).

Potassium salt of *p*-aminobenzoic acid (KPAB) is to be favored over sodium salt for the treatment of dermatomyositis. It is less likely to cause anorexia or nausea. It will not enhance the edema which is commonly present in patients with dermatomyositis. The compound is best administered as 10% aqueous solution. Both these preparations will darken on exposure to ultraviolet light, and the solutions of KPAB should be kept in a dark place, preferably refrigerated. KPAB is administered orally, usually 3 gm., 30 cc. of a 10% solution, every three hours, for six doses per day, in adult cases. Patients may take the solution straight or follow it with a small amount of water, milk, ginger ale, or soda. The dosage is maintained at 18 gm. daily during the first weeks of therapy; improvement is gradual but progressive. As soon as satisfactory progress has been obtained, the dosage of KPAB may be reduced to 12 gm. per day. The therapy is usually required for months and then may be stopped. If relapse occurs, it has to be started again.

Phenylbutazone may be of value in the therapy of the systemic and ocular signs of rheumatoid arthritis (Gsell and von Rechenberg; Brodie). But there is evidence that although phenylbutazone has some effects similar to cortisone, its action is probably not through the production of adrenal corticoids (Breck; Brodie). Phenylbutazone, like cortisone and corticotropin, does cause retention of sodium, chloride, and water and may reactivate peptic ulcers. Unlike cortisone, however, it does not affect the excretion of potassium nor does it cause eosinopenia or increased ketosteroid excretions. Phenylbutazone, however, must be used with caution because of its side-effects. Frequent reactions are water

retention, nausea, rash, epigastric pain, vertigo, and stomatitis. Unusually severe reactions include peptic ulcer activation with bleeding, hepatitis, hypertension, transient psychosis, moderate leucopenia, agranulocytosis, thrombocytopenia, and purpura without thrombocytopenia. The patients showing agranulocytosis usually recover after discontinuance of medication. Whenever this drug is used, the patients must be carefully supervised because of these frequent side-reactions (Kuzell; Nathan; O'Brien and Storey).

Summary of Systemic Varieties of Therapy

It is evident from a review of the literature that the most important group of agents in the therapy of collagen diseases consists of the steroid compounds. These include corticotropin, cortisone, hydrocortisone, prednisone, and prednisolone. A variety of other systemically administered agents have been tried and are of some value. These include the salicylates, phenylbutazone, transfusions, anticoagulants, gold therapy, roentgen therapy, and surgical excision in specific cases, such as excision of a piece of the temporal artery in temporal arteritis and excision of the malignancy which seems to be associated with some types of collagen disease, and one might include desensitization for those cases where definite hypersensitivity has been demonstrated but the allergens cannot be removed from the environment. One must be aware of these other agents, in addition to the steroids, for unfortunately steroids are not always successful in controlling the disease process. This is particularly true in temporal arteritis, where there have been a number of instances of failure to control the disease by the use of these agents (Anderson and Vallotton). In one case observed at the Wills Hospital steroid therapy plus excision of the temporal artery plus anticoagulant therapy failed to bring about any significant improvement in the disease process or in its ocular effects. Many instances of failure in other forms of collagen disease such as scleromalacia perforans and lupus erythematosus can be given for each and all of these agents.

Wise found transfusions to be of temporary benefit in two cases of periarteritis nodosa.

Anticoagulants may be used for the occluded retinal vessels. Their value has not been decided definitely (Anderson and Vallotton; Maumenee).

Gold therapy has been of some help in rheumatoid arthritis. It is not very helpful in chronic ankylosing spondylitis or Marie-Strumpell arthritis, but roentgen irradiation to the spine does sometimes bring about benefit in the latter type. This does not seem to aid the associated uveitis where steroid therapy frequently fails.

Chrysotherapy.—The treatment of rheumatoid arthritis with gold was introduced by European physicians in 1927. At present corticotropin and cortisone seem to offer more rapid means than gold for relieving symptoms of this condition. Sometimes corticotropin or cortisone has not been helpful and gold may still be used. Both agents can be combined in therapy. Dimercaprol (BAL) is effective therapy for toxic manifestations which occasionally result from gold treatment.

All preparations of gold are given intramuscularly. There is no reason for intravenous administration. Gold sodium thiosulphate (Sanochrysin) or gold sodium thiomalate (Myochrysine) possess 37% to 50% gold content, respectively, in aqueous solution. One usually begins at 10 mg. doses, which are in-

creased progressively to 25 mg. Injections are usually given twice weekly and are continued until the patient has received 1 to 1.5 gm. of the salt. Then maintenance doses of 10 mg. are given weekly or twice monthly for four to six months. Toxic reactions from this therapy are very possible and should always be mentioned to the patient prior to its administration. They may vary in incidence from 20% to 80%, according to various reports.

The most frequent toxic manifestations affect the skin and mucous membrane. They may range from mild pruritis or stomatitis to intense exfoliative dermatitis, peripheral neuritis, and encephalitis uveitis, and renal lesions have been reported, as well as effects on the hemopoietic organs producing leucopenia, agranulocytosis, hemorrhagic purpura, and hemolytic anemia. The dangerous toxic signs may appear without any preliminary warning, although a complete check of the blood cell count, particularly the platelets, has been done on all patients. These toxic features do respond to the administration of dimercaprol. This comes in 10% solution of peanut oil containing 20% of benzyl benzoate. It is administered intramuscularly at intervals of four to six hours for one or two days and then twice daily for six to eight days, then once daily until the last evidence of toxic reactions has disappeared. The dose is 2.5 mg/kilogram body weight.

Local Therapy.—Local therapy of these conditions includes the use of steroids. Available for local use are hydrocortisone, cortisone, prednisone, prednisolone, and fludrocortisone. These agents are available as the acetate or free alcohol forms and can be applied in suspensions or in ointment vehicles. The frequency of instillation will be determined by the severity of the condition and the response to initial therapy. Usually these therapies have to be continued for long periods of time in order to bring about remissions and to prevent relapses.

In addition to the anti-inflammatory agents, such as steroids, one must employ other nonspecific local measures such as cycloplegics for the dilatation of the pupil and the rest of the eye when the uveal tract and cornea are involved.
same purpose.

Vasodilations have been employed locally retrobulbarly for vascular occlusions associated with collagen disorders. Anticoagulants are used systemically for the

The handling of each ocular complication in a nonspecific manner with local therapy is well known to all ophthalmologists and need not be defined here. There is nothing that differentiates the therapy of uveitis of collagen disease from the uveitis of other nonspecific etiologies or that of keratitis, optic neuritis, retinopathy, periphlebitis, etc.

Complications of Therapy.—All these agents have undesirable side-effects and toxic actions with which one must be familiar and constantly alerted to their development in the patient. One should not use drugs with dangerous side-effects for self-limiting diseases. This applies to a disease such as Reiter's disease, which we know is self-limited and which may be included in this group of conditions which involve the eye. For example, gold therapy has been suggested as treatment for this condition. The toxicity of this drug, however, hardly justifies its use in such a self-limited disorder.

Summary

It is apparent that the therapy of collagen diseases which produce ocular manifestations is not completely satisfactory. Only in those instances where one is fortunate enough to uncover an etiologic factor is there room for any feeling

of security concerning the disorder. Such an instance would be the development of lupus erythematosus after the use of hydralazine (Apresoline). This type of lupus seems to disappear with the cessation of therapy. The polyangiitis nodosa following the use of sulfonamides may also disappear with removal of the exciting agent. The clearing of collagen disease following the removal of malignancy is another example of favorable therapy. Most discouraging are the lupus erythematosus cases that fail to respond to any form of therapy, scleromalacia perforans that progresses in spite of all forms of treatment, temporal arteritis, polyarteritis nodosa, erythema multiforme, and others which may continue in spite of treatment. The chances of therapy improving will depend on research which will uncover other specific causes for each of these conditions. Perhaps as we understand more about the metabolism of connective tissue and its reaction to the various stimuli our therapy will become more specific and helpful.

BIBLIOGRAPHY

Anderson, B., and Vallotton, W.: Etiology and Therapy of Retinal Vascular Occlusions, A.M.A. Arch. Ophth. 54:6, 1955.

Bayliss, R. I. S., and Steinbeck, A. W.: Salicylates and the Plasma Level of Adrenal Steroids, Lancet 1:1010, 1954.

Breck, E.; Fearmely, N. E.; Meacock, I., and Patley, H.: Phenylbutazone Therapy: Relation Between Toxic and Therapeutic Effects of Blood Level, Lancet 1:225, 1954.

Brodie, B. B.; Loman, E. W.; Burns, J. C.; Lee, T. R.; Chenkin, D.; Goldman, A.; Warner, M., and Steele, J. N.: Observations on Anti-Rheumatic and Physiologic Effects of Phenylbutazone (Butazoladine) and Some Comparisons with Cortisone, Am. J. Med. 16:181, 1954.

Comparison of Cortisone and Aspirin in the Treatment of Early Cases of Rheumatoid Arthritis: Joint Committee of the Medical Research Council and Nuffield Foundation on Clinical Trials of Cortisone and ACTH; Therapeutic Measures in Chronic Rheumatoid Diseases, Brit. M. J. 1:1223, 1954.

Done, A. K.; Ely, R. S., and Kelley, V. C.: Studies of 17-Hydroxycorticosteroids: III. Blood Levels in Salicylate Intoxication, J. Pediat. 44:153, 1954.

Dubois, E. L.: Quinacrine (Atabrine) in the Treatment of Systemic and Discoid Lupus Erythematosus, A.M.A. Arch. Int. Med. 94:131, 1954.

Ehrich, W. E.: Nature of Collagen Diseases, Am. Heart J. 43:121, 1952.

Gsell, O., and von Rechenberg, H. K.: Combined Therapy of Chronic Polyarthritis with Cortisone and Butazolidine, Schweiz. med. Wchnschr. 83:1079, 1953.

Kierland, R. R.; Brunsting, L. A., and O'Leary, P. A.: Quinacrine Hydrochloride (Atabrine) in the Treatment of Lupus Erythematosus, A.M.A. Arch. Dermat. & Syph. 68:651, 1953.

Klemperer, P.: Collagen Disease, editorial, Am. J. Med. 10:405, 1951.

Klemperer, P.: Concept of Collagen Disease, Am. J. Path. 26:505, 1950.

Kornblueth, W.; Laufer, A.; Lochbel, E., and Frisher, M.: Fibrinoid Degeneration of the Tarsal Conjunctiva, A. M. A. Arch. Ophth. 49:671, 1953.

Kuzell, W. C.; Schaffarzick, R. W.; Naugler, W. E.; Gaudin, G., and Mankle, E. A.: Phenylbutazone: Further Clinical Evaluations, A.M.A. Arch. Int. Med. 92:646, 1953.

Lansbury, J.: Connective Tissue Manifestations of Neoplastic Disease, Geriatrics 9:319, 1954.

Harvey, A. M.; Shulman, L. E.; Tumulty, P. A.; Conley, C. L., and Shoenrich, E. H.: Systemic Lupus Erythematosus: Review of the Literature and Clinical Analysis of 138 Cases, Medicine 33:291, 1954.

Maumenee, A. E.: Diseases of the Retina, A.M.A. Arch. Ophth. 49:675, 1953.

Nathan, D. A.; Meitus, M. L.; Capland, L., and Lev, M.: Death Following Phenylbutazone (Butazoladin) Therapy, Ann. Int. Med. 39:1096, 1953.

O'Brien, D. J., and Storey, G.: Death from Hypersensitivity Due to Phenylbutazone, Brit. M. J. 1:792, 1954.

Reinhardt, D. J., and Waldron, J. M.: Lupus Erythematosus-like Syndrome Complicating Hydralazine (Apresoline) Therapy, J.A.M.A. 155:1491, 1955.

Smith, M. J. H.; Gray, C. H., and Lumnon, J. B.: Urinary Excretion of Adrenocortical Steroids by Patients Receiving Salicylates, Lancet 1:1008, 1954.

Soffer, L. J.; Elster, S. K., and Hamerman, D. J.: Treatment of Acute Disseminated Lupus Erythematosus with Corticotropin and Cortisone, A.M.A. Arch. Int. Med. 93:503, 1954.

Spies, T. D.; Stone, R. E.; García López, G.; Díaz Tellechea, C. M.; López Toca, R.; Reboredo, A., and Suarez, R. M., Sr.: Prednisone and Prednisolone as Therapeutic Agents: Progress Report on Their Integration into General Medical Practice, J.A.M.A. 159:645, 1955.

Stillerman, M. L.: Ocular Aspects of Diffuse Collagen Disease, in Modern Trends in Ophthalmology, Vol. 3, edited by A. Sorsby, New York, Paul B. Hoeber, Inc. 1955, p. 158.

Tye, N. J.; Scheff, B. L.; Collins, S. F.; Baylor, G. R., and Appel, B.: Chronic Discoid Lupus Erythematosus Treated with Daraprim and Chloroquine Disphosphate (Aralen), New England J. Med. 51:52, 1954.

Vail, D.: Diffuse Collagen Diseases with Ocular Complications, Tr. Ophth. Soc. U. Kingdom 72:155, 1952.

Wiesel, L. L., and Barritt, A. S.: Long-Term Treatment of Rheumatoid Arthritis with Para-Amino-Benzylic Acid and Cortisone Acetate, Am. J. M. Sc. 27:74, 1954.

Wise, G. N.: Ocular Periarteritis Nodosa: Report of 2 Cases, A.M.A. Arch. Ophth. 48:1, 1952.

Zarafonetis, C. J. D.; Steiger, W. A.; Ginsburg, I. W., and Haether, A. J.: Treatment of Rheumatoid Arthritis with P-Aminobenzoate and Acetylsalicylic Acid, A.M.A. Arch. Int. Med. 92:204, 1953.

Annual Reviews

PHYSIOLOGICAL CHEMISTRY OF THE EYE

A Review of Papers Published During 1955

ZACHARIAS DISCHE, M.D., New York

1. Cornea

CONSIDERABLE progress has been reported in 1955 from studies on the nature of the proteins of the cornea and of the corneal metabolism as well as on the controlling effect of the metabolism of the corneal epithelium and endothelium on the hydration of the cornea, first suggested by Davson and Maurice and experimentally supported by the studies of Smelser and Ozanics and of Langham.

François and Rabaey [1] investigated the proteins in the corneal epithelium of beef and hog. The scraped-off epithelium was homogenized, and the proteins were precipitated at −10 C with acetone or extracted with veronal buffer at pH 8.6 or phosphate buffers of pH 7.2, 6.2, and 5.3. The acetone powder was dissolved in water. The proteins were separated by paper electrophoresis. Fraction 2 comprised 65% of total protein and was found to have a slightly lesser mobility than the β-lipoprotein fraction of serum run simultaneously. The mobility of Fraction 1, which corresponded to 12% of total protein, was between that of α_1- and α_2-globulin. Fraction 3, corresponding to 9% of the total, had a mobility identical with that of γ-globulin, and Fraction 4, corresponding to 6.5% of the total, was somewhat behind the latter. No lipoproteins were found by Sudan black I staining. The periodic acid-Schiff reaction according to Hotchkiss showed Fraction 3 to be a glycoprotein.

Münich and Oswald,[2] using paper electrophoresis according to Grassmann and Hannig in veronal buffer of pH 8.6, also found in the corneal epithelium four different fractions. The authors used materials from calves' eyes and centrifuged their homogenates of corneal epithelium 45 minutes at 4500 rpm. The supernatant was subjected to electrophoresis during 12 to 14 hours. The quantitative ratios of the four fractions listed according to decreasing mobility were 3.63%, 20.96%, 59.55%, and 15.86%. These values differ completely from those reported by François and Rabaey. A better agreement, at least as far as the order of magnitude of the quantities of different fractions is concerned, would appear if the Fraction 3 of the authors would be identical with Fraction 2 of François and Rabaey and Fraction 2 would be identical with Fraction 1 of the latter authors. Münich and Oswald stress also the lack of identity between the protein fractions of the corneal epithelium and those of the blood plasma of the same species. This difference does not appear surprising, but according to Demling it does not appear in the case of liver proteins.

Van Den Hooff [3] reported the results of electron microscopic studies of the corneal connective tissue on preparations called by the author replicas. He found that the cornea consists of layers of parallel fibrils only one fibril thick. The subsequent layers of fibers run at varying angles. The periodicity of these collagen

Received for publication June 22, 1956.

fibers became apparent only after removal of the mucoid, which was carried out by either enzymatic or mechanical means. The diameter of the fibrils was found, in agreement with other investigators, to be about 350 A. The periodicity of the collagen fibers of the cornea, however, was found to be only 210 A; that means only one-third of what is usually found with other collagen fibers.

Dohlman and Balazs [4] made an extensive study of the composition of Descemet's membrane of the cornea of beef. The Descemet membranes were carefully completely separated from the cornea stroma. Although on the basis of chemical analysis the organic material must be regarded as collagen, the electron microscopic analysis and the solubility show great differences between the material of the Descemet membrane and other collagenous tissues. The latter contained 13.8% to 14% of dry weight nitrogen. It contained 73% of the dry weight collagen when this was determined by extraction first at 4 C with 10% $CaCl_2$ and then with H_2O. Of the total nitrogen, 9.8% corresponds to hydroxyproline, as compared with 13% in corneal stroma. On the basis of ultraviolet absorption the collagen of the Descemet membrane contains 6.4% tyrosine and 1.9% tryptophan, compared with 1.5% and 4.5%, respectively, in the corneal stroma. The chemical determination by the Millon reaction according to Bernharts gave only half the values for tyrosine in the Descemet membrane as well as in stroma. The collagen fibers of the Descemet membrane were found to contain a neutral polysaccharide with only 2% hexosamine and the three hexoses, galactose, glucose, and mannose, present in the ratio 6:2:1. The total amount of the reducing substances determined after hydrolysis of the fibers corresponded to 11% to 13% of sugar in terms of glucose equivalents.

Dohlman and Boström [5] investigated the rate of exchange of sulfate with the acid mucopolysaccharide of the cornea and of the sclera. Carrier-free S^{35}-labeled Na_2SO_4 was injected intraperitoneally into 40 white rats (200 to 220 gm.). The animals received 2μc per gram. The animals were killed after time intervals varying between 8 hours and 17 days. The cornea and sclera were separated from the rest of the eye and freed of inorganic sulfate by repeated washing with one-third saturated Na_2SO_4 and distilled water. Control experiments in which corneas were first soaked in radioactive Na_2SO_4 showed that no significant amounts of inorganic radioactive sulfate remained in the preparations. The experimental samples were then dried, and their radioactivity was measured directly. It has been shown in control experiments in which the experimental samples were hydrolyzed in 6 N HCl for four hours and liberated sulfate was precipitated as $BaSO_4$ that the direct measurements of the radioactivity carried out on the experimental samples gave sufficiently correct values. The incorporation of SO_4 into the mucopolysaccharides reached a peak 24 hours after the injection and began then to go down, reaching about 70% of the maximum in about 100 hours and then rising again slightly before further declining very slowly so that the half life of the radioactivity was not reached even after 17 days. In the sclera, on the other hand, the peak of the radioactivity was already reached after 16 hours and then declined continuously, reaching half of its maximum value after 10 days. This behavior is in good agreement with the corresponding data for skin chondroitin sulfate and somewhat shorter than for cartilage chondroitin sulfate (17 days). The authors give two tentative explanations for the much slower exchange of sulfate with the mucopolysaccharides of the cornea than with those of the sclera. One is that the keratosulfate, which is present only

in the cornea, has a much slower rate of exchange. The other is the avascularity of the cornea.

Schiff [6] identified chromatographically as cystine crystals found in the corneas and conjunctivas of two patients with cystinosis. Dinitrophenyl derivatives of cystine were used for chromatography.

Cagianut [7] reported data on the chemical composition of the Kayser-Fleischer cornea rings in two cases. The pigment of the ring was found soluble in KCN and strong acid, insoluble in alkali. Spectroscopy revealed presence of Hg, Cu, and Ni, although according to the authors no silver medication took place. Much smaller amounts of copper were found in normal corneas. The authors assume that the disease which is characterized by hyperaminoaciduria is a disturbance of the protein metabolism with subsequent derangement of the heavy metal metabolism.

Kinoshita and co-workers [8] carried out an important investigation on the glucose metabolism in corneal epithelium of beef. Scraped-off corneal epithelium, 700 to 800 mg., was incubated for three hours at 37 C with glucose labeled with C^{14} either in carbon 1 or in carbon 6. The radioactivity of the respiratory CO_2 and of the produced lactic acid was determined. It was found that 6.7 times as much labeling was present in CO_2 when glucose labeled in Position 1 was used as substrate than when glucose labeled in Position 6 was used. On the other hand, the corresponding ratio in the produced lactic acid only was 0.65. The sum of the radioactivity incorporated in CO_2 and lactic acid was identical with both forms of labeled glucose, which indicates that no significant amounts of intermediates were accumulated. Of the total glucose, 65% can be assumed, on the basis of these results, to be broken down to lactic acid via the glycolytic pathway and 35% to be broken down oxidatively almost exclusively by the direct oxidative pathway, only a very small part over the tricarboxylic acid cycle. While these experiments of the authors certainly suggest a major role of the direct oxidative pathway of glucose metabolism in the corneal epithelium, it is not yet clear whether the same preponderance of this pathway indicated by their experiments on scraped-off corneal epithelium from beef is also valid in vivo. It must be noted that de Roeth has shown in our laboratory that the oxidative metabolism of the corneal epithelium when investigated while still connected with the stroma is characterized by a high Meyerhof quotient, and no lactic acid is produced under aerobic conditions. The scraped-off epithelium, on the other hand, according to de Roeth, shows a considerable aerobic glycolosis. This suggests that the corneal epithelium in situ could at least partly operate through the tricarboxylic acid cycle.

Cogan and Kuwabara * studied the lipogenesis by cellular elements of the cornea from injected oleate or oleic acid and blood serum. They found accumulation of fat in corneal cells surrounding the necrotic site of injection by staining with Sudan and Nile blue. The optimum pH of this lipogenesis was between 6 and 7.8. None occurred below 4.95 and above 8.5. Lipogenesis occurred also under nitrogen but was eliminated by NaF and 1% KCN. Addition of serum was shown to be necessary. The essential factor in serum was not removed either by ether extraction or heating to 60 C for one hour. Stearic acid and stearates produced only a barely significant lipogenesis. No lipase was demonstrable in the cornea by the Gomori technique after incubation with either stearic

* References 9 and 10.

or oleic acid. The cellular elements producing fat were fibrocytes. Later foam cells appeared, which were found to be derived from wandering cells entering the stroma from the limbus.

Iida [12] made histochemical studies of the development of the phosphatase in the cornea. He investigated the influence of hyaluronidase, epinephrine, insulin, cortisone, injury, and storage in vitro on this enzyme. During embryonic development, a maximum of the phosphatase activity in the cornea was found on the 13th day.

Gloster [11] determined in homogenized rabbit corneas the carbonic anhydrase and found its content in the cornea to be 400 times less than that in blood cells. In the epithelium and endothelium it was 1/100 and 1/400 of the concentration in blood cells, respectively. The concentration of this enzyme is, therefore, much lower in the cornea than it is in the lens and in the iris.

The mechanism of the hydration of the cornea, particularly in relation to the cornea metabolism, has been the subject of several extensive studies. Davson [13] compared the hydration of the cornea kept for periods of 17 to 23 hours in a moist chamber at 7 C with that of eyes kept at 31 C in an atmosphere of air which passed through 0.9% NaCl solution. The air under these conditions was found not to be completely saturated with water vapor. While the water content of cornea maintained at 31 C was found to be 77.5% ±0.14, the corneas from eyes maintained at 7 C were found to contain 82.2% ± 0.3 water. When eyes after incubation at 7 C were kept for six to eight hours at 31 C, a reversal of hydration was obtained. Incubation of eyes in nitrogen atmosphere instead of air at 31 C resulted in a strong increase in hydration. The amount of water per gram of dry weight in the corneas was under these circumstances more than 100% higher than in corneas of the contralateral eyes kept in air. Scraping off of the epithelium from the corneas resulted in an increase of hydration of the corneas at 31 C. The effect of the scraping off was much smaller at 7 C. The hydration due to refrigeration could be partly reversed by subsequent incubation at 31 C. These experiments support the view that, at least in vitro, the hydration of the cornea is regulated by metabolic processes in the epithelium and endothelium.

Langham [14] arrives at identical conclusions on the basis of studies on the hydration of excised rabbit corneas kept in Krebs-Ringer phosphate solution at 37.5 C under nitrogen and oxygen, respectively. The hydration was measured by the increase in thickness, which after incubation of five hours was 0.44 mm. in O_2 and 0.54 mm. in N_2 atmosphere, respectively. The hydration was accompanied by an increase in NaCl concentration. Cooling at 4 C caused swelling even in O_2 atmosphere. This was reversed by a reincubation under O_2, but not under N_2, at 37 C.

Similar studies were carried out by Harris and Nordquist.[15] They studied the effect of refrigeration on hydration as well as on the content in Na and K of the cornea when whole eyes were kept in moist chambers for varying periods of time at 37 and 4 C, respectively. By comparing the amount of water with the amount of the two cations entering the cornea when the eyes were incubated at 0 C they found that after 20 hours 20% of the hydration was due to the entering of pure water and 80% to the movement of aqueous humor. When the period of refrigeration of the eyes at 0 C for 45 hours was followed by incubation at 37 C, water moved out of the cornea more rapidly than sodium. If the period of refrigeration lasted only 20 hours, the subsequent incubation at 37 C showed

during the first few hours loss of water without loss of sodium. The dehydration by incubation at 37 C after refrigeration did not take place under anaerobic conditions. The authors interpret these results as a demonstration of the regulatory effect of the metabolism, particularly of the endothelium, on the maintenance of the state of hydration of the cornea in vivo.

Philpot [16] also studied the influence of various chemical factors on the hydration of rabbit corneas. The hydration of the cornea was studied in eyes which were incubated in moist chambers at 32 C for 16 to 24 hours. Into the aqueous of one eye of an experimental animal was injected KCN, iodoacetic acid (IAA), NaF, dinitrophenol (DNP), various dyes, or anticholinesterase drugs like atropine, while the other eye served as control. IAA at a concentration of 10^{-4}, NaF at 2.5 to 3.3×10^{-2} M, and DNP at 1.2 to 1.7×10^{-2} M and basic dyes increased the hydration of the cornea. KCN below 10^{-2} did not. This, however, could be due to an elimination of HCN in the tissues. Anticholinesterases were without effect. The authors believe, therefore, that the effect of these drugs on the hydration of other tissues is a purely physical phenomenon, due to the absorption of these drugs on cellular surfaces and not due to the inactivation of cholinesterase.

The problem of the significance of corneal metabolism for the hydration of the cornea was approached in a somewhat different way by Smelser and Chen.[17] These authors studied the effect of contact lenses on the thickness, optical density, and lactic acid production of guinea pig corneas. The lenses were worn for periods between one-quarter and six hours, after which time the animals were killed and the corneas were dissected under cooling. The thickness was measured by caliper, and the turbidity of the cornea was measured with a Beckman spectrophotometer after the corneas were mounted on a methacrylate (Lucite) base. The corneas of contralateral eyes served as controls. The corneas wearing contact lenses were found to contain up to 150% more lactic acid after six hours than the controls. This was paralleled by an increase in optical density and in thickness of the cornea, while the content in nitrogen remained almost constant. These results support the concept that under conditions of deficient aerobiosis, as manifested by the increased lactic acid production, the cornea is unable to maintain its normal state of hydration. The same authors [18] approached the problem of the regulation of corneal hydration from still another interesting angle. They compared the histology of the cornea, degree of swelling in various media, and its dependence on various factors and the chemical composition of the mucoid in a fresh water fish (carp) and in guinea pigs. This comparative study, the results of which were reviewed on the basis of the paper given at the 17th International Congress of Ophthalmology last year in the ARCHIVES, appears interesting because the cornea of the fresh water fish does not have a lacrimal film and is always surrounded by a hypotonic solution.

Another important factor for the hydration of the cornea and the permeability of the epithelium was studied by Maurice.[19] He measured the penetration of Na^{24} through the cornea into the aqueous by test solutions of NaCl and $Na^{24}Cl$ during a period of nine and one-quarter minutes. The concentration varied between 0.25% and 10%, and the pH, from below 4 to above 10. Hypertonic solutions were found not to affect the permeability of the epithelium; hypotonicity increased it. Variations of the pH from 4 to 10 were without effect. The intactness of the epithelium in these experiments was tested by fluorescence.

François and Rabaey [20] made histochemical studies of the relation of the acid mucopolysaccharides of the cornea to swelling of this tissue. Phase microscopy and electron microscopy were used. It was shown that above pH 4.5 only the mucopolysaccharide participates in the swelling. When vascularization was induced by alloxan or corticosterone, the content in mucopolysaccharides, evaluated by metachromatic dying with methylene blue, increased. The increase, however, was only relative and was explained by the simultaneous swelling of the cornea. Serum albumin in 5% concentration and serum itself inhibit in vitro the swelling of the cornea. The inhibition is stronger when epithelium is present.

Grant and Kern [21] made an interesting systematic study of the binding of various cations from alkaline solutions by corneal collagen. Solutions with a concentration of 0.23 M were dropped into rabbit conjunctivas after the corneal epithelium was scraped off during a period of 15 minutes. The pH varied between 11 and 12. Excised corneas of beef, rabbit, and man were exposed to salt solutions of pH 12. Collagen was found to accumulate cations in the ratio of 10:1 from the solution at a pH between 11 and 12 but not to bind them at pH 7. Earth alkalis eliminated K and Na from the equilibrium. Corneal mucoid, on the other hand, combined with cations at pH 7. The binding of calcium could be reversed only by rinsing of the cornea for two days. Rinsing with edathamil disodium (Versene) in 0.1 M solution prevented severe injury by calcium only when the injury was not deep-seated. Rinsing with 0.1 M HCl made the injury worse. Treatment of the corneal stroma with $Ca(OH)_2$ for 15 minutes at pH 11 increased by 200% to 300% the extractability of mucoid by water.

Guidry and co-workers [22] studied the effect of acid injury of the cornea on the ascorbic acid content of the cornea and the aqueous. The cornea was injured by dropping every 10 seconds for 1 minute two drops of 1 N HCl in the conjunctiva and rinsing with H_2O for 15 minutes. Starting with 24 hours after the injury, the ascorbic acid in the cornea decreased one-third to one-half for a period of 120 hours. In the aqueous during the same period it decreased by 42% in the anterior but not in the posterior chamber. No utilization of the ascorbic acid by the burnt cornea was found during one hour at 37 C. The authors conclude, therefore, that the decrease in the ascorbic acid is due to a higher permeability of the iris vessels for this substance.

DeSimone [23] investigated the oxygen consumption and anaerobic glycolysis of the rabbit cornea in experimental neuroparalytic keratitis.

Sakai [24] found a decrease of pH in infected and allergic inflammation of the cornea.

Wiesinger and co-workers [25] reported that riboflavin deficiency can be a contributing factor in the vascularization of the cornea and that high-protein low-fat diet has a protective effect against this damage.

Rokhlina and Zubarova [26] studied the effect of pantothenic acid and of vitamin B_6 on the regeneration of the corneal epithelium.

Gandolf and Montaldi [27] found that the antiepithelizing effect of hydrocortisone can be eliminated by application of chlorophyll.

Gezurian and Persoglia [28] studied the effects of cortisone acetate on the corneal epithelium of the guinea pig.

Passmore and King [29] have reviewed research on vital staining of conjunctiva and cornea.

Lewke [30] made an attempt to trace the way by which fluid penetrates and flows out of the cornea by injecting $FeCl_2$ into the conjunctiva and $K_3Fe(CN)_6$ in the aqueous and determining the formation of a colored precipitate in the cornea. Under normal conditions the precipitate appeared as a peripheral ring starting from the limbus. When the central part of the cornea was rinsed with distilled water, however, it appeared across the rinsed area but not in the area covered by the lids. The author concludes that the water transported into and out of the cornea is regulated by the epithelium and that tears and aqueous are necessary for its integrity. These conclusions do not appear convincing on the basis of the experimental material.

2. Aqueous Humor

During the current year a number of significant contributions to the study of the dynamic of the formation of the aqueous have appeared which in general support the concept of the dynamic of the aqueous flow developed by Kinsey and Friedenwald. These investigations were mainly based on studies of the difference in the concentration of certain key constituents of the aqueous, like Na, bicarbonate, and ascorbic acid, between the anterior and posterior chamber and on the study of the effect of the carbonic anhydrase inhibitor acetazoleamide (Diamox) on the intraocular pressure and on the aqueous. In addition, a number of significant studies was concerned with the homoeostatic mechanisms which regulate the intraocular pressure by influencing the aqueous flow.

Wislocki and Ladman [31] made an attempt to determine the site of the blood ocular barrier in the albino rat by adding to the drinking water of four male rats 0.15% of $AgNO_3$ during a period of 10 to 13 months. Ag deposits were found in the basement membrane of the ciliary body, indicating a blood ocular barrier at this site. A blood-retina barrier was found in Bruch's membrane as well as in the ground substance surrounding the retinal capillaries. The possibility, however, of chemical interaction between silver and proteins lets the identification of the site of the barrier by this method appear doubtful.

The nature of the protein in the aqueous was investigated by Rao and his associates [32] by electrophoresis as well as by Oudin's gel diffusion technique. The first method indicated the presence of three proteins, the electrophoresis patterns of which appeared identical with those of lens proteins. The immunological method indicated the presence of six proteins, three of which appeared identical again with lens and three with serum proteins. The authors assume a diffusion of lens proteins and products of their protolysis into the aqueous, which would explain the high value of NPN in the dialyzed aqueous.

Keup and Steiger [33] determined by two-dimensional paper chromatography the compositition of the amino acids of the aqueous and found that the relative concentrations of the amino acids of the aqueous agree with those of serum except as far as glutamic acid and lysine are concerned. The former was found in higher, the latter in lower, concentration. Butanol glacial acetic acid and water and cresol phenol were used as solvents for chromatography, borate buffer for extraction from the paper, and ninhydrin for the quantitative determination of amino acids.

A series of extensive studies concerning the mechanism of the formation of the aqueous have been carried out during the current year. Davson [34] determined the steady state ratio of aqueous to plasma concentration in rabbits for a large

number of ions and nonelectrolytes as well as the rate of penetration of these substances into the aqueous. The data thus obtained were compared with the data simultaneously obtained for the distribution and penetration into the cerebrospinal fluid in the same animals under the same experimental conditions. The variations in different species were studied in horse, dog, cat, guinea pig, and rabbit, and the effect of age on the distribution of chloride was studied by comparing the values in 3- to 5-month-old puppies and in calves of 10 to 28 days of age with those of adult animals. The steady state in these experiments was obtained by initial intravenous injection of 10 ml. of isotonic solution and maintained by continuous infusion with a variable-speed injection machine. The steady state distribution and the rate of penetration were measured either by determining radioactive ions Na^{24}, K^{42}, Br^{82}, I^{131}, or by chemical determinations of $CaNS^{-}$, Cl^{-}, thiourea, methylthiourea, ethylthiourea, propylthiourea, ethyl alcohol, creatinine, glucose, sucrose, and *p*-aminohippurate. The effect of penetration of these substances into the lens and vitreous was investigated by analyzing the concentration in these two tissues. The data obtained were subjected to a mathematical treatment under certain simplifying assumptions, and in the light of kinetic studies and the steady-state distribution ratios the conclusion is reached by the authors that the primary process in the formation of the fluid is an active transport of sodium from plasma to the posterior chamber.

In another study, Davson [35] examined the validity of Kinsey and Palm's claim that thiocyanate and Na penetrate into the aqueous by different mechanisms. To this end the distribution of thiocyanate between plasma and plasma dialysate at different concentrations of this anion varying between 0.40 and 6.35 mM. was examined, and the adsorption of this anion to protein demonstrated. The ratio of nonadsorbed thiocyanate to total thiocyanate at 2.83 mM. was found to be 0.75. This adsorption phenomenon, according to the author, can explain the differences between the penetration of thiocyanate and Na into the aqueous found by Kinsey and Palm.

Friedenwald and Becker [36] subjected the dynamics of the formation of the aqueous to an extensive mathematical treatment based on the equation derived by Kinsey and Palm which expresses the rate of change of concentration of a test substance in the anterior chamber in relation to inflow and outflow from the chamber and diffusional exchange. The authors examined first to which extent certain arbitrary assumptions made during the derivation of the equation limit the validity of this equation. These assumptions concern the degree of the utilization of the test substance in the ocular metabolism, the degree to which samples of the fluid from the posterior chamber are representative of the fluid passing through the pupil, the exchange of test substance between anterior chamber and cornea, the outflow from the anterior chamber in a bulk, and that no significant fluid enters the anterior chamber except via the pupil. The authors found that the differential equations used by Kinsey and Palm may be considered valid, and they construct on the basis of these equations a more general theory of the dynamic of the aqueous humor.

The role of carbonic anhydrase in the regulation of the composition of the aqueous has been the subject of numerous investigations which have used the inhibiting effect of acetazoleamide on this enzyme as a tool. Lee [37] investigated the effect of the inhibition of carbonic anhydrase on the pH, bicarbonate, and CO_2 concentrations in blood and aqueous humor. He found a decrease of pH

and bicarbonate. In normal conscious rabbits the pH, the bicarbonate, and the CO_2 were found to be higher in the aqueous than in the blood, namely, 7.8 ± 0.018 compared with 7.67 ± 0.011 mM/kg. H_2O; 30.82 ± 1.24 compared with 22.74 ± 0.89 mM/kg. H_2O, and 0.89 ± 0.051 compared with 0.68 ± 0.025 mM/kg. H_2O, respectively. After an acetazoleamide injection of 100 mg/kg. and hourly intravenous injections of 50 mg/kg. as 10% Na salt, a new steady state was reached after 25 hours with a pH of 7.7 ± 0.14 compared with 7.6 ± 0.01; 22.2 ± 0.29 bicarbonate compared with 16.35 ± 0.46 mM/kg. H_2O, and CO_2 concentration of 0.82 ± 0.028 compared with 0.59 ± 0.022 mM/kg. H_2O.

Gloster [38] found a significant fall in ocular pressure after intravenous injection of only 5 mg/kg. acetazoleamide in four rabbits. Injections into the anterior chamber or conjunctiva were without effect.

Davson and Luck [39] report other observations on the distribution of bicarbonate between aqueous humor and plasma in several species. The ratio of aqueous to plasma concentration of bicarbonate is in an inverse relation to the size of the animal in the series guinea pig, rabbit, cat, dog, monkey, goat, and horse. The authors suggest that the higher rate of secretion of bicarbonate is necessary for the neutralization of the lactic acid and pyruvic acid by the retina and lens, the relative mass of which as compared with the vitreous increases with decreasing size of the globe.

Langham [40] found that acetazoleamide increased the time of appearance of fluorescein in the anterior chamber from 48 ± 26 to 348 ± 21 seconds. Acetazoleamide, 50 mg/kg. was injected before the appearance of fluorescein. The drug reduces the rate of flow of the aqueous, but it does not affect the permeability of the blood aqueous barrier.

Becker and Constant [41] measured the alteration in rabbit aqueous flow after inhibition of carbonic anhydrase by acetazoleamide. Rabbits received 100 mg/kg. of the drug, and the aqueous flow was determined by tonography with an electronic tonometer. The tonography was carried out before injection and 45 to 60 minutes after injection. In a series of 50 eyes an average systemic inhibition of 63% of the flow was found. In some rabbits no corresponding decrease in intraocular pressure could be found. The authors explain the negative results, as far as the influence of acetazoleamide on intraocular pressure is concerned, as due to a compensatory shutting down of the outflow mechanism in certain rabbits. In preliminary experiments it was found that systemic administration of adrenal steroid prevents this compensatory decrease in outflow facility, but the changes of aqueous flow induced by acetazoleamide are not significantly altered by these steroids. This increase in the facility of outflow also explains why the suppression of aqueous flow lasts longer than the decrease of intraocular pressure.

Becker [42] made an attempt to correlate the effect of acetazoleamide on the secretion of aqueous humor with changes which this drug induces in the bicarbonate and ascorbic acid content in the posterior and anterior chambers of the eye. Albino rabbits and guinea pigs were used for these experiments. The animals received the drug, 100 mg/kg., intravenously plus 25 mg/kg. every 30 minutes for 6 hours. Nephrectomized animals received a single dose of 100 mg/kg. intravenously. The steady state with ascorbic acid was achieved in nephrectomized animals with 500 mg. of ascorbic acid orally, while non-nephrectomized animals received 50 mg. subcutaneously every 30 minutes for 6 hours. The concentration

of bicarbonate was calculated from the directly determined concentration of CO_2, assuming the pH values of 7.4 for rabbit plasma and 7.55 for the aqueous. Possible changes of the pH by the drug were disregarded. The average decrease by acetazoleamide of posterior chamber bicarbonate was 8.4 mM/liter, and the decrease was from an excess over plasma of 68% down to 38%; in the anterior chamber these values were 7.2 mM/liter and from 36% to 10%. The application of the simplified formula of Kinsey and Friedenwald to these steady state data led to an estimate of 64% decrease in aqueous flow, in agreement with the estimate previously obtained by tonography. The concentration of ascorbic acid six hours after acetazoleamide administration rose 50% in the posterior chamber and 14% in the anterior chamber. Similar rises took place in animals with elevated ascorbic acid plasma levels. The authors discuss three possible mechanisms which can explain this rise in ascorbic acid and find that it is in agreement with the theory of dynamics of the aqueous flow proposed by Kinsey and Friedenwald.

Kinsey and his associates [43] independently carried out a similar investigation on bicarbonate and ascorbate in the aqueous and in addition studied the effect of acetazoleamide on the concentrations of chloride and sodium. While the changes of the first three substances conformed to their theory of the aqueous flow, they found that the turnover rate of sodium increased instead of decreasing as the theory predicted. They point out that these findings indicate a need for reevaluation of the secretion diffusion theory of aqueous humor formation as far as the mode of entrance of sodium is concerned.

Appelmans [44] found after oral administration of acetazoleamide an increase in the protein content of the aqueous humor without change in the volume of the aqueous. The proteins were determined by precipitating 0.1 cc. of the aqueous with 0.1 cc. of 20% trichloroacetic acid (TCA) and estimating the volume of the precipitate after 24 hours. They explain the increase in proteins by an increase in permeability of the barrier. Acetazoleamide had no effect on the protein content of the secondary aqueous.

Falbriand and his associates [45] found after acetazoleamide injection a decrease in the level of potassium (15.3% of the normal average value) but no change in the concentration of sodium. The same authors [46] also found a slight decrease of the pH of the aqueous in rabbits after 100 mg/kg. of acetazoleamide intravenously. They report that the statistical analysis of their data shows the changes to be significant.

Sugar and Fainstein [47] studied the effect of acetazoleamide (500 mg/kg. orally) on the outflow from the aqueous in normal persons and in patients with operated on and nonoperated on glaucoma of various types. They used tonographic measurements with a Miller electrotonometer after the water test. The rate of flow dropped in normal eyes from 1.92 to 1.52 microliters (μl.) per minute, in nonoperated on eyes with simple glaucoma from 1.71μl. to 1.50μl., and in operated on eyes with simple glaucoma from 2.30μl. to 1.52μl.

Two groups of investigators reported findings concerning effects of acetazoleamide which appeared to be in disagreement with those of other investigators. Weinstein and Forgáes [48] found that the decrease of $NaHCO_3$, pressure, and pH after acetazoleamide administration is not accompanied by increased fluorescein permeability. Acetazoleamide acts by increasing the reabsorption of the aqueous. Epinephrine injection was found to decrease the secretion. Acetazoleamide was ineffective in absolute glaucoma.

Green and co-workers [49] studied the significance of carbonic anhydrase for the maintenance of intraocular tension. They measured the intraocular pressure of rabbits every 30 minutes during 3 hours with a Schiøtz tonometer and the carbonic anhydrase activity of homogenates of the ciliary body before and after intravenous injection of 30 to 100 mg/kg. of acetazoleamide or 100 mg/kg. of *p*-carboxybenzenesulfonamide or 5 to 20 mg. and 10 mg. of the two drugs, respectively, subconjunctivally. No effect on the ocular pressure was observed, although the carbonic anhydrase is reported to be completely inhibited. Special experiments were carried out to ascertain that the inhibition of the enzyme was achieved in vivo and was not due to contamination with the inhibitor from blood during homogenization. The authors conclude that the carbonic anhydrase is not involved in the regulation of the intraocular pressure.

Green and co-workers [50] investigated the role of carbonic anhydrase on the bicarbonate level in the aqueous. Intravenous injections of 30 to 100 mg/kg. of acetazoleamide increased the bicarbonate in the plasma temporarily but did not significantly affect its concentration in the aqueous 30 minutes after the injection. Bicarbonate was measured in the aqueous by the Warburg technique after saturation of the fluid with an atmosphere of 95% N_2+5% CO_2. The authors conclude that the carbonic anhydrase is not necessary for the elaboration or maintenance of bicarbonate in the aqueous. These data contradict Lee's data obtained 20 to 50 minutes after injection. The latter author unfortunately does not report in his brief communication details of his experimental procedures.

Friedenwald [51] disposes of objections against the possible role of carbonic anhydrase in the aqueous secretion processes based on negative results obtained by Green and his associates. These negative results, as far as the influence on intraocular pressure and the rate of inflow are concerned, are explained by the fact that the carbonic anhydrase is present in large excess, so that even a few per cent of the active enzyme can still adequately perform its physiological function. As far as the negative results on the concentration of bicarbonate of the aqueous are concerned, they are explained by the inadequacy of the methods used by Green and co-workers, particularly as far as statistical evaluation of results is concerned. Friedenwald reviews all the current investigations on the effect of acetazoleamide on the intraocular pressure and the concentration of various constituents of the aqueous and points out that all the available data can be predicted on the basis of the secretion-diffusion theory and the redox hypothesis of the formation of the aqueous. He stresses the importance of homoeostatic mechanisms which tend to compensate for the decrease in the outflow of the aqueous after the inhibition of carbonic anhydrase by an increase in the resistance to outflow, demonstrated in the maintenance of the intraocular pressure after injection of *p*-aminohippuric acid in rabbits in spite of the diminished rate of flow of the aqueous and a similar phenomenon after carotid ligation. He discusses the role of these homoeostatic mechanisms in the nonresponsiveness of certain rabbits to acetazoleamide and in the phenomenon that the decrease in ocular pressure lasts a shorter time than the decrease in aqueous flow.

Friedenwald [52] attempts an explanation of the role of carbonic anhydrase in the production of the aqueous based on experiments carried out in collaboration with Becker on the effect of acetazoleamide on the intraocular pressure. He points out that, on the basis of tonographic experiments of Becker and his own experiments on the effect of acetazoleamide on the permeability of the blood aqueous

barrier for fluorescein, it can be assumed that acetazoleamide affects the influx without affecting the outflow. The role of carbonic anhydrase is assumed to be the regulation of the transfer of hydrogen ions from the stroma to the epithelium of the ciliary body, which, according to the redox hypothesis of the intraocular secretion, is mediated by ascorbic acid. The inhibition by carbonic anhydrase therefore results in the acidification of the stroma and alkalinization of the epithelium, which may interfere with the activity of enzymes involved in the secretary process. This interpretation appears supported by the fact that in rabbits made refractory to acetazoleamide by suboptimal medication the elimination of the refractory state by bicarbonate is not affected by simultaneous administration of ascorbic acid, while the latter counteracts the effectiveness of NH_4Cl in restoring the sensitivity to acetazoleamide.

Green and his associates [53] investigated the concentration of acetazoleamide in various ocular tissues after intraveneous injection into rabbits. They developed a new spectrophotometric method for the determination of acetazoleamide based on the increase of the ultraviolet absorption of the aqueous at 290 mμ induced by acetazoleamide. Determinations can be carried out on 0.1 cc. of aqueous. A concentration of 23γ/cc. of acetazoleamide was found in aqueous 60 minutes after intravenous injection of 30 mg/kg., 30 to 90 minutes after subconjunctival injection of 15γ to 190γ/cc. Determination in iris and ciliary body tissue by the enzymatic method resulted in 5γ to 8γ per ciliary body and iris after intravenous injection.

Ballintine and Maren [54] investigated the concentration of acetazoleamide in the iris and in ciliary processes after intravenous injections of 10 to 20 mg/kg. into rabbits, as well as the activity of carbonic anhydrase in these tissues. They compared in this respect rabbits which responded normally to acetazoleamide with nonresponsive ones to determine whether nonresponsiveness may be due to differences either in the activity of the enzyme or in the amount of the drug which penetrates into the ocular tissues. No differences between the two groups of animals were found as far as these two factors are concerned.

An interesting contribution to the problem of varying responsiveness of rabbits to acetazoleamide was made by Kinsey and his co-workers.[55] They defined a responsive animal as reacting with a decrease of intraocular pressure (IOP) of 3 scale divisions of the Schiøtz tonometer, using 5.5 gm. weight 30 to 60 minutes after 100 mg/kg. of acetazoleamide intravenously. Their experiments are based on the observation that the rabbit diet (Rockland farms diet) in their laboratory, in which rabbits were mostly nonresponsive, contained only one-sixth of the amount of sodium present in diets used in laboratories with responsive animals. In experiments on 35 young albino rabbits adequate addition of NaCl to the Rockland diet increased their responsiveness, as did giving 0.2 NaCl in drinking water. The effect of the diet was reversible, and NaCl did not induce any significant changes in the pH or in the concentration of Na, K, Cl, and CO_2 of the blood. Aldosterone injected 60 minutes before the administration of acetazoleamide also increased responsiveness of 10 rabbits from 2 to 4 scale divisions. This effect, however, was so rapid that it cannot be explained by the salt-retention effect of the steroid. No explanation for these changes of responsiveness can be given at present.

In view of this effect of aldosterone, the study of Green and his associates [56] is of particular interest. The authors first determined the concentration of steroids

in the aqueous of rabbits before and 20 minutes after paracentesis. They found 1.7γ/ml. in the first and 8γ/ml. in the second case. The method of analysis was the tetrazolium blue method of Mader and Buck modified by the authors. In a second investigation [57] the nature of this steroid was determined by paper chromatography. A larger volume of pooled samples of aqueous was extracted with 5 volumes of chloroform. This extract, concentrated in vacuo, was chromatographed with toluene-propylene glycol as solvent, which was allowed to run down completely. A standard of hydrocortisone was used simultaneously. The chromatogram sprayed with the blue tetrazolium-NaOH reagent showed only one spot exactly coinciding with the spot given by the standard. The same spot on a parallel chromatogram extracted with methanol showed in ultraviolet light an absorption curve identical to that of the standard. The dry residue of the methanol extract treated with concentrated H_2SO_4 produced a chromogen with an absorption curve identical to that of hydrocortisone. The quantitation of the chromatogram yielded a value of 3γ hydrocortisone per 17 ml. of aqueous. The authors report that even one-third of this quantity of cortisone would have been detectable with their chromatographic procedure. They consider, however, the possibility that cortisone acetate, which, owing to its higher R_F value, could be present in the overflow of the solvent, might be also a constituent of the aqueous.

The ineffectiveness of the acetazoleamide after local application was reported by Foss,[58] who injected into rabbits 0.1 ml. of 1% to 10% solution of acetazoleamide subconjunctivally and 1% intracamerally without any effect on the intraocular pressure. These findings are in agreement with those of Gloster and Perkins [59] on rabbit and cat eyes and with those of Green and Leopold,[60] who injected subconjunctivally 10 mg. of acetazoleamide in 0.2 ml. H_2O and did not find any significant change in the bicarbonate concentration in the posterior chamber 30 to 60 minutes after injection. Nor did they observe any lowering of the IOP. The authors point out that previous investigations have shown that measurable quantities of the drug were found in the anterior uvea 90 minutes after subconjunctival injection of 10 mg. of acetazoleamide and that the carbonic anhydrase appeared inhibited in in vitro experiments. They do not exclude, however, the possibility that the inhibition did not occur in vivo. The authors observed also in these experiments an effect of acetazoleamide on the permeability of the blood aqueous barrier, demonstrated by the fluorescein test and increased protein concentration of the aqueous.

Kinsey and his associates [61] determined the rate constants of the flow of the aqueous due to diffusion and to the flow from the posterior to the anterior chamber for SCN^- and Na^+, respectively. They found the following figures:

SCN^-	K diffusion	0.0085 to 0.010
	K flow	0.017 to 0.020
Na^+	K diffusion	0.0145
	K flow	0.017

Friedenwald [62] determined the rate of flow of the aqueous by determining the concentration of ascorbic acid in the posterior and anterior chambers at varying levels of the substance in plasma. From 0.4 to 4 gm. of ascorbic acid injected subcutaneously into nephrectomized rabbits yielded a constant plasma level of 20 to 60 mg. per hour. Twenty hours later the ascorbic acid was determined in the anterior and posterior chambers. At 90 mg. per 100 cc. of ascorbic acid in plasma the anterior chamber-plasma ratio as well as the posterior chamber-anterior chamber ratio was 1. With changes in the concentration of ascorbic acid in plasma

there were corresponding changes of the concentration in the anterior chamber. This shows diffusion between anterior chamber and blood and no significant utilization of ascorbic acid, which allows the use of ascorbic acid for determination of flow. Friedenwald obtained 2.2% of volume per chamber and minute, somewhat faster than Kinsey's data obtained with thiocyanate and Na.

The significance of the back diffusion of the ascorbic acid from the aqueous into the blood appears also in observations by Böhringer and Schelling,[63] who found that while the ascorbic acid in human aqueous can reach a maximum of 20 mg. per 100 cc. when the saturation level of 1 mg. per 100 cc. prevails in serum, in inflammatory processes of the eye the aqueous level reaches only 16 mg. per 100 cc.

Bárány and Langham [64] investigated the relation between the concentrations of ascorbic acid in the plasma and aqueous of guinea pig and rabbit, with emphasis on the possibility of synthesis of ascorbic acid in the eye. They also carried out experiments to determine the possible importance of the dehydroascorbic acid in the blood as precursor of the ascorbic acid in the aqueous. They determined ascorbic acid by a modification of the osazon technique of Held and Langham, which they found agreed in its results with the usual 2,6-dichlorophenol-indophenol method with the aqueous of normal but not that of scorbutic animals. They determined the correlation between the ascorbic acid levels in the aqueous and the levels in the blood in scorbutic as well as nonscorbutic guinea pigs and rabbits before and after injections of ascorbic acid which assured a steady state in the plasma. An analysis of the correlation between plasma and aqueous levels does not give a conclusive answer as to whether ascorbic acid is formed in eye tissues but suggests that in the rabbit a concentration in the aqueous of 14 mg/100 ml. prevails at a 0 concentration in the plasma. In the guinea pig the saturation level in the plasma was found to be only 0.8 mg/100 ml. This value is much lower than that reported by Linnér, which the authors explain by the inadequacy of the method of determination used by the latter author. There was no clear-cut correlation between the preformed dehydroascorbic acid level in plasma of rabbit (0.09±0.01 mg/100 ml.) and the ascorbic acid level of the aqueous. No convincing evidence for the presence of dehydroascorbic acid in plasma of guinea pigs was found. Experiments with injection of dehydroascorbic acid into the blood showed in rabbits an increase in aqueous ascorbic acid. No evidence for a conversion of dehydroascorbic acid into ascorbic acid in ocular tissues was found. In the guinea pig dehydroascorbic acid injections decreased the ascorbic acid of the aqueous. This unexpected effect can not be explained at present.

Langham [65] carried out experiments of the same kind. He determined ascorbic acid in aqueous of normal rabbit eyes and compared it with the level of ascorbic acid in the contralateral eye after ligation of the corresponding carotid. The ratio of ascorbic acid in the eye operated on to that in the eye not operated on was about 0.80. An ascorbic acid level in plasma of 3 to 5 mg. per 100 cc. (determined by 2-dichlorophenol indophenol) was found as saturation level on the side not operated on, and a level of 6 to 8 mg. per 100 cc., for the eye on the side operated on. The ascorbic acid level in aqueous at saturation was equal on the two sides. The data agree with those foreseen from estimated decrease of plasma flow after carotid ligation.

The effect of ascorbic acid on the steady state aqueous to plasma ratio of Na^+ and Cl^- was studied by Ridge [66] in normal animals and subclinically scorbutic animals, which after 10 to 12 days of scorbutogenic diet showed no ascorbic acid in the aqueous. Increase of plasma ascorbic acid to saturation levels (12 mg. intraperitoneally followed every half hour by 3 mg.) had no effect on the steady state ratio of the eyes. The author finds in these data a support for Bárány's views against those of Friedenwald.

Auricchio reviews in three papers † current investigations on the physiology and dynamic of the intraocular fluid.

A series of papers deals with homoeostatic regulations of the aqueous flow. Bárány [70] reported briefly that carotid ligation which, according to Linnér, decreases the inflow of the aqueous, also increases the angle resistance to outflow, while increase in the blood pressure in the episcleral veins has the opposite effect. These effects may contribute to the maintenance of the normal IOP in spite of variations in blood pressure.

Bárány and Woodin [71] reported the results of an extensive investigation on the so-called massage effect and effect of injection of hyaluronidase on the decrease of the resistance to outflow of aqueous through the anterior chamber angle of the rabbit. The purpose of this investigation was to obtain indications about the nature of the substance affected by hyaluronidase and the possible role of this enzyme in the regulation of the angle resistance to the outflow of the aqueous. Enucleated cattle eyes were perfused with NaCl barbital buffer as well as cattle aqueous for various periods of time, according to the previously described technique of perfusion. The possibility was extensively examined that the massage effect may be due to a liberation of an active substance in the massaged eye. It was not possible, however, in these experiments to achieve a breakdown of resistance in a nonmassaged eye by the aqueous of a massaged one. The massage effect could be achieved also in eyes containing 0.12 mg. per milliliter of diethyldithiocarbamate, which chelates Cu^{++} in the anterior chamber. While a normal massage effect was observed also in the eyes of calves, three pig eyes proved resistant to massage. Staphylococcal hyaluronidase was found very effective in breaking angle resistance to outflow. In a series of experiments it was found that while the concentrated aqueous is able to depolymerize hyaluronic acid, the process is of not enzymatic origin and no significant amounts of hyaluronidase could be detected in the aqueous, which was found to contain between 20γ and 80γ of hyaluronic acid per milliliter. Massage did not increase the depolymerizing activity of the aqueous. The depolymerizing activity of the aqueous was also tested on the hyaluronic acid protein complex from synovial fluid. It was found that the viscosity-reducing action is more thermostable than that of commercial preparations of hyaluronidase, is diffusible, and can be completely inhibited by the addition of 250γ per milliter of sodium diethyldithiocarbamate. These properties appear similar to those of the substances which, according to Skanse and Sandblatt, degrade hyaluronic acid by oxydation. The authors conclude from these experiments that the massage effect comes about through an immediate physical derangement of the tissue of the angle followed by a slower temperature-dependent phase which is not inhibited by diethyldithiocarbamate. The effect of hyaluronidase which could be obtained by the staphylococcal enzyme appears also diphasic, as, in addition to the immediate action of hyaluronidase in low concentrations, this enzyme

† References 67-69.

in high concentrations induces a further decrease of the resistance of the angle. After the drop in resistance induced by massage the resistance is found to be less affected by hyaluronidase. Massage after hyaluronidase had no effect at all, which indicates that the point of attack of massage is identical with that of hyaluronidase.

The presence of a nonenzymatic mechanism of depolymerization of hyaluronic acid by a reducing substance in the aqueous and uvea of beef is also reported by Tagaki.[72]

Friedenwald [52] reports that the decrease of the aqueous inflow after administration of *p*-aminobenzoic acid does not lead to a fall in IOP and suggests that this is due to a concomitant increase in the angle resistance.

Homoeostatic effects due to regulations by the autonomous nervous system or drugs affecting this system have been reported by various investigators. Sutcliff and Hamilton [73] noticed two minutes after paracentesis of one eye a decrease in the concentration of ions in the contralateral eye, which after four minutes gave place to an increase. The first phenomenon is interpreted as due to an inflow of water; the second, to a compensatory nervous regulation of the secretory process.

Auricchio [74] found that the bicarbonate concentration in the secondary aqueous is higher than that in plasma but lower than that in the normal aqueous. His attempt to influence aqueous secretion by pilocarpine-acetylcholine and eserine had doubtful results.

Seki [75] found that subconjunctival injections of epinephrine, but not that of pilocarpine or atropine, impeded the transition of urea from serum to aqueous. The transfer of urea from aqueous to blood was found to be slow. No urease was found in eye tissues.

According to Ito,[76] pilocarpine instillation decreases the pyruvate level in aqueous, probably owing to an increase in outflow. Atropine had no effect.

According to Inamochi,[77] eserine and pilocarpine increase the permeability of the blood-aqueous barrier for fluorescein. Subconjunctival injection of epinephrine impeded transfer across the barrier. Removal of the ciliary ganglion increased the pilocarpine effect. Atropine and novocaine injected retrobulbarly had no effect.

Viikari [78] studied the cholinesterase activity of the aqueous in man and animals and the influence of anticholinesterases on it. The first part of his investigation deals with determinations of the cholinesterase activity of aqueous in man, rabbit, cat, cow, horse, pig, and sheep. By biological methods, using m. rectus abdominis of the frog and the dorsal muscle of the leech for the determination of the acetylcholine content, it was possible to demonstrate that almost without exception the aqueous humor of above-mentioned animals showed cholinesterase activity, although the amounts are small. No essential differences between the values for the various species of animals were found. The author also obtained confirmation of the fact that the cholinesterase activity in the secondary aqueous is greatly increased. Probably a greater part of the cholinesterase of the rabbit and cat is nonspecific. In the second part of the investigation the author studied the influence exerted on the cholinesterase activity of the aqueous by reversible anticholinesterases, i. e., physostigmine and neostigmine, and by certain of the new anticholinesterases of the polyalkylphosphate group, i. e., isoflurophate (DFP), tetraethylpyrophosphate (TEPP), hexaethyltetraphosphate (HETP), a diethyl-

p-nitrophenyl phosphate (Mintacol), and tabun (dimethylamino-ethoxy-phosphoryl cyanide). The latter have chiefly an irreversibly inhibiting action on cholinesterase. He further showed that physostigmine and neostigmine either inactivate the cholinesterase in aqueous humor or produce no demonstrable change. On the other hand, the cholinesterase activity of aqueous humor is frequently clearly increased by polyalkylphosphate, even in the usual therapeutic concentrations. In low concentrations either no change or slight inactivating action was found. The increase in cholinesterase activity is probably the result of increased capillary permeability, which is a known effect of all anticholinesterases, and particularly of polyalkylphosphates. The flow of proteins into the anterior chamber caused by the change in permeability presumably also conveys with it such a large amount of cholinesterase that the instilled anticholinesterase, even a powerful one, is not adequate for its inactivation.

A regulatory effect of substances derived from the pituitary gland was demonstrated in a series of investigations. Ross [79] studied the effect of intravenous injections of 2 ml. of crude anterior pituitary extract, containing 2 to 7 mg/ml. of growth hormone, on the permeability of the blood-aqueous barrier, using the transfer of glucose as a measure. The injections increased the transfer constant from 1.89 to 5.1 to 5.6. When 2 and 10 units of Novoinsulin (insulin free of glucagon) were injected one hour before the injection of the growth hormone, the effect of the latter remained unchanged. Although the injection of 2 units of Novoinsulin alone increased k_{in} to 4.8, this increase was almost completely eliminated by subsequent injections of 10 units more of Novoinsulin. This shows that the effect of the growth hormone is not due to the mobilization of insulin.

Kishimoto and co-workers [80] found that extract from the anterior pituitary (Antuiturin) increases the thiamine content of the aqueous and vitreous. Chorionic gonadotropin (Antuiturin S) is more active than the growth-promoting principles (Antuiturin-Growth).

Schmerl and co-workers ‡ found substances in the posterior and anterior pituitary and in the cerebrospinal fluid which, according to these authors, have a specific regulatory effect on the maintenance of the IOP. Cerebrospinal fluid contains a substance, hyperpiesin, which, injected intravenously, increases IOP. Injections of varying amounts of spinal fluid into four rabbits with IOP varying between 21 and 33 mm. Hg increased it after 30 to 40 minutes. The square variation of the tension in normal eyes was only ± 1 mm. The animals displayed symptoms of acute glaucoma after the injection. When the spinal fluid is incubated for 70 minutes at 37 C with a crude extract of the pituitary or with vasopressin (Pitressin) preparations and then injected intravenously, it causes a depression of the IOP of up to 14.5 mm. Dialyzed preparations of pituitary extract retain their effectiveness. This indicates that the extracts contain a nondialyzable catalyst, perhaps an enzyme, which converts hyperpiesin into another substance, called miopiesin by the authors, which has the opposite effect on the IOP. The authors report that rabbits with congenital glaucoma do not possess this catalytic substance in the pituitary and spinal fluid. Spinal fluid without hyperpiesin activity incubated with extracts from the anterior pituitary shows hyperpiesin activity. The effects of miopiesin grow with the dose, and depressions of up to 6 mm. Hg have been observed. Extracts of the anterior and posterior pituitary alone have no effect whatsoever.

‡ References 81 and 82.

An effect of substances related to allergic phenomena in the body on the permeability of the blood-aqueous barrier has been observed by several investigators. D'Ermo [83] finds an increase in the permeability of the barrier in anaphylactic shock.

Chodos [84] reports that phenylbutazone (Butazolidin), 30 mg/kg. injected intravenously for 10 days preceding reinjection of horse serum, counteracts the anaphylactic decrease of the permeability. Synopen (2-[*p*-chlorobenzyl (2-dimethylaminoethyl) amino] pyridine) (20 mg/kg.) 30 minutes before serum reinjection has no effect. Intravenous administration of 4-butyl-1,2-diphenyl-3,5-pyrazolidinedione (Irgapyrine) (100 mg/kg.) decreases in 30 to 45 minutes the permeability of the barrier measured by the fluorescein test, according to Amsler and Huber.

Küchle § found a loosening effect on the barrier by aminosalicylic acid (PAS), and the penetration of this substance into the aqueous was facilitated only by intravenous injection of pilocarpine and cocaine. Also, intravenous injections of hyaluronidase were effective in this respect. The permeability for PAS was also increased after experimentally induced inflammation of eye tissues in rabbits.

Steiger and associates [87] determined proteins in secondary aqueous obtained at different time intervals between 30 minutes and 48 hours after paracentesis. They found significant increase up to several hundred per cent 30 minutes after paracentesis. Electrophoretic studies showed small but significant changes in the relative amounts of α and β globulins.

3. Lens

Considerable progress was made during 1955 in the chemistry of lens proteins and in the elucidation of the mechanisms involved in the lenticular metabolism.

Francois and associates [89] carried out an extensive study on the fractionation of lens proteins. They used a new method for fractionation based on principles elaborated in E. Cohn's laboratory for the fractionation of blood plasma proteins. Fractionation was carried out separately on the cortical and nuclear parts of lenses of cattle. The lens homogenates, containing about 5% of soluble proteins, were contrifuged to separate the albuminoid, and the soluble proteins were fractionated by ethanol precipitation, varying the pH and ionic strength of the solution as well as the concentration of ethanol and the temperature. Cortical homogenates were first adjusted to pH 5 with N/10 HCl and thereafter with N/10 NaOH. This precipitated a fraction designated as α_1 crystalline. The precipitation of the supernatant with 13% ethanol at 5 to 10 C yielded a second fraction designated as α_2 crystalline. Traces of the latter fraction were then removed by increasing the ethanol [2] to 17%. The supernatant was then adjusted to pH 8 with NaOH and 1/10 M barbital (Veronal); the ionic strength was increased by adding NaCl to 0.5%, and the proteins were precipitated at −10 C with 26% ethanol. The precipitate was split by electrophoresis into two components called Fractions 2 and 3, corresponding to 56.5% and 4.5% of total cortex proteins, respectively. The two α crystalline fractions were electrophoretically undistinguishable. The application of basically the same procedure to nuclear proteins revealed significant differences in the solubility as compared with that of cortex proteins and different ratios between the individual fractions. Thus, the adjustment of the

§ References 85 and 86.

homogenate to pH 5 precipitated all α crystalline and the Proteins 2 and 3 were precipitated already at −4 C by 13% ethanol. The ratio of fractions 3 to 2 was strongly increased in the nucleus. Molecular weight determinations by light scattering yielded for the cortical α_1-crystalline fraction a value of 200,000 and a value of 1,200,000 for the α_2 fraction. Electromicroscopic studies indicated a much higher degree of polymerization of the cortical protein fractions.

The authors claim that their fractionation procedure yields protein fractions in a more native state than previously used procedures, but their report does not contain any evidence for that claim, and the separation of the crystalline fraction into two subfractions differing in their solubility was already achieved by salt fractionation by Burky and Wood and the electrophoretic separation of β crystalline into two different fractions can be achieved by prolonged electrophoresis of preparations obtained by salting-out procedures.

The same authors [90] investigated changes in soluble lens proteins with varying pH and found that Fractions 1 and 2 in lenses of cattle are denatured below pH 3.5 and above pH 9. Fraction 3, on the other side, was much more resistant and was not significantly denatured between pH 3.2 and 10.5. The irreversibility of precipitation was used as criterion for denaturation. The authors point out that small changes of pH as are found in cataractous lenses cannot be responsible for changes in proteins.

Szent-György [91] reported interesting observations on the fluorescence of the protein fraction from bullock lenses. The lenses were stored for two months at −20 C before the preparation was carried out. The protein called by the author lentoglobulin has the character of a euglobulin, and it is precipitated from distilled water at pH but is soluble in diluted salt solution. Its amount corresponds to about one-third of the total proteins of the lens. Its brilliant blue fluorescence is lost after heating, and lentoglobulin itself is easily denatured, particularly when kept in an ammonium sulfate solution, from which it is precipitated at 37% saturation. It appears necessary to integrate these findings on the physical properties of lentoglobulin with what is known about the physical properties of lens fractions of cattle reported by other authors.

Stemmermann and Wallner [92] carried out electrophoretic studies on the composition of soluble proteins of the lens of the chick during embryonic development. They found that a protein with the mobility of α-crystalline appears much earlier than β-crystalline.

The fractionation of the insoluble albuminoid of the lens of cattle was carried out by Fromageot and co-workers.|| The albinoid was obtained by homogenizing lenses in 1/10 M phosphate buffer of pH 7.6 at 0 C and washing the residue with water. One fraction was then obtained by extracting the albuminoid prepared from 100 gm. lens with 150 ml. formamide at 0 C and centrifuging for one hour at 0 C at 20,000 rpm. The supernatant was precipitated with three parts of cold acetone, and the precipitate was dissolved in 150 ml. 1/200 N NaOH with a final pH of 12. This fraction 1 corresponded to 25% of the original albuminoid and contained 0.5% of sulfur. The fraction was uniform in the ultracentrifuge and was precipitated almost completely at a pH of 5.3 and by $(NH_4)_2SO_4$ at pH 7.3 at 25% of saturation. A second fraction was obtained by extracting the residue which was remaining after extraction of Frac-

|| References 93 and 94.

tion 1 with N/200 NaOH with N/10 NaOH at 0 C for several hours. This fraction precipitated after dialysis against 0.1 M NaCl. It was uniform in the ultracentrifuge and also contained 0.5% sulfur. A third fraction with a higher sulfur content, of 0.85%, could be obtained from the residue of the formamide extraction of the original albuminoid by adding 5% glycerol to formamide. It was precipitated by three parts of ether and dissolved in N/10 NOH. A fourth fraction represented the residue insoluble in formamide plus glycerol. It also contained 0.85% sulfur. Thus study demonstrates the inhomogeneity of the albuminoid and seems to exclude the possibility that the albuminoid in lenses of cattle is formed by direct transformation of one of the soluble proteins.

Fromageot and Prioux [95] investigated the proteolytic activities of the lens. In confirmation of older data of Krause, they found two different proteases, with optima at pH 6.7 and 7.2, respectively. But they made the important observation that cysteine or ascorbic acid is necessary for a significant activity of these enzymes. In a lens homogenate in 1/10 M phosphate buffer corresponding to a 100-fold dilution of the lens a significant proteolysis was observed at 37 C in 70 minutes when 0.032 M of cysteine was present in solution. The authors expressed the interesting idea that the loss of reducing substances in the lens with progressing senility may inactivate lens proteases and lead to a degeneration of lens proteins which cannot be rejuvenated by resynthesis.

Wieme and Kaminski [96] determined the composition of the soluble proteins of the cattle lens by two immunological methods, namely, a modification of the gel diffusion method of d'Ouchterlony and the immunoelectrophoresis method of Grabar and Williams. The immune serum was prepared by injection of extracts of lens nucleus into two rabbits (two intraperitoneal injections of 2 ml. of a 5% solution of soluble nuclear lens proteins for three months). While the gel diffusion method yielded only three immunologically distinct protein fractions, the immunoelectrophoresis yielded eight different antigenic fractions. Rao and coworkers,[32] by Oudin's immunological gel method, found only six immunologically characterized protein fractions in the lens.

The fine structure of the lens capsule was investigated by electron microscopy by Van Den Hooff.[3] Several layers of parallel fibers were found which were only about 50 A. thick and were held together by a homogeneous viscons substance. The chemical nature of the fibers was not established.

Ujejski and Glegg [97] identified tentatively by paper chromatography the saccharides present in the lens capsule. In addition to glucose and galactose, found previously by other authors, they also report to have found spots on the paper chromatogram corresponding to mannose and xylose. As the quantitative relations between these sugars were not determined, it is difficult to interpret these findings. It seems most probable that the two sugars which were not found previously by other investigators as part of the fibrous material of the capsule may come from capsule constituents present in very small amounts which were removed from preparations of the fibrous material of the capsule used by other authors.

The aerobic and the anaerobic metabolism of the lens itself and of the lens capsule were the subject of a number of investigations. Kinoshita [98] investigated the relative importance of the tricarboxylic acid cycle and of the so-called direct oxidative pathway of glucose oxidation in the lens. To this end he added

to rabbit lenses, suspended in balanced salt solution in vitro, glucose which was labelled with C^{14} in either the carbon 1 or the carbon 6 position and compared, on the one hand, the content, on C^{14}, of respiration CO_2 in both cases with, on the other hand, the amount of labeling in lactic acid produced in both cases. It was found that about 40 times as much labeling was recovered in the CO_2 when glucose labelled in Position 1 was used as in the other case. This indicates that respiration via the tricarboxylic acid cycle plays only a minor role in the oxidative metabolism of the lens of rabbit and is replaced by the direct oxidative pathway which produces ribulose-5-phosphate from glucose-6-phosphate G-6-P by decarboxylation of the carbon 1. In the lactic acid, on the other hand, the labeling corresponded only to 21% of the total, from which it could be calculated that about one-fifth of the glucose metabolized by the lens is broken down oxidatively, while four-fifths of it is glycolized. It is noteworthy that the ratio between the oxidative and the glycolytic breakdown of glucose in the lens arrived at by the isotopic method is in complete agreement with the ratio originally found by the direct measurement of the lactic acid production and oxygen consumption in the rabbit lens in vitro made by Kronfeld. Kinoshita could also demonstrate the synthesis of the mixture of glucose- and fructose-6-phosphate and sedoheptulose monophosphate from ribose-5-phosphate added to rabbit lens homogenates. Although the reaction products in these experiments apparently were only identified tentatively by analytical methods, it seems remarkable that an almost stoichiometric relation was obtained between the amount of ribose, which disappears, and the above-mentioned reaction products. These experiments on homogenates support the concept of the predominance of the direct oxidative pathway of the glucose oxidation in lens. As the oxidation of glucose-6-phosphate to ribose-5-phosphate requires the reduction of TPN, the problem arises how the reduced TPN is reoxidized. Kinoshita revives the concept, first introduced in 1924 by Goldmann and Adams, of the metabolic significance of ascorbic acid and glutathione in assuming that these compounds catalyze the oxidation of $TPNH_2$ by O_2. He shows that the reduction of glutathione by the glutathione reductase of the lens can be coupled with the reduction of TPN by G6P and that glutathione increases the direct oxidation of G6P by 68% and ascorbic acid by 40%.

Mandel and Klethi [99] demonstrated the presence of the glucose-6-phosphate and 6-phosphogluconic acid dehydrogenases in homogenates of calf lenses. In these experiments the lenses were extracted with a fourfold volume of cold distilled water and the activity of the enzymes was measured spectrophotometrically by the reduction of added TPN in 1/10 M phosphate buffer of pH 7.1. The turnover of glucose-6-phosphate in these homogenates equals that of phosphogluconic acid.

The mechanism of glycolysis in the lens was subject of three penetrating studies by Green and his associates.¶ They studied the anaerobic metabolism of glucose-6-phosphate (G6P) and fructose-1,6-diphosphate (FDP). The rabbit lenses were homogenized in a fourfold volume of a balanced solution of pH 7.5. These lens extracts did not display any significant adenosine triphosphatase (ATPase) activity, which made it possible to accumulate certain intermediate products of glycolysis and to obtain satisfactory balances between oxidoreductive processes of glycolysis and phosphorylation. It was found that the concentration

¶ References 100-102.

of hexokinase in these extracts is a limiting factor for the turnover of glucose, while the amount of phosphate acceptors limits the turnover of G6P. The breakdown of glucose, in addition, proceeded only as long as inorganic phosphate was available. Arsenate, however, as well as myosin and adenosine diphosphate also increased lactic acid production from glucose. Adenosine triphosphate (ATP), as well as adenosine monophosphate (AMP) increased the turnover of G6P. On the other hand, FDP was only very slowly broken down by the lens extract produced. However, considerable amounts of lactic acid were formed when either ATP or AMP, which produce adenosine diphosphate (ADP), was added or when arsenate was added, which eliminates the necessity of a phosphate acceptor. These results are in complete agreement with the operation of the Embden-Meyerhol glycolytic pathway in the anaerobic glycolysis of the lens. This conclusion is borne out by a further investigation by the authors on the metabolism of triosephosphate, phosphoglycerate, and phosphoenolpyruvate. Phosphoglycerate added to the extract produced lactic acid only when reduced diphosphopyridine nucleotide (DPN) was added. Under the conditions of the experiment an amount of phosphorus liberated by hydrolysis for seven minutes in 1 N HCl at 100 C [7′ P] equivalent to the amount of lactic acid was produced. Without $DPNH_2$ 24% of the added phosphoglycerate was found converted to phosphoenolpyruvate, which about corresponds to the ratio of the two compounds at equilibrium. The phosphate transfer from phosphoglycerate to AMP or ADP was inhibited by NaF. The authors also studied the relation between the synthesis of 7′P and oxidation of triosephosphate and found that about two atoms of P disappeared when one mole of triosephosphate was oxidized. The basis of these calculations, however, in these latter experiments is not quite clear, as the authors do not describe their procedure in determining the turnover of triosephosphate. The latter apparently is in equilibrium in the lens extract with FDP, and the total turnover of triosephosphate, therefore, would have required the determination not only of the triosephosphate, which disappears, but also of the changes in the content of FDP.

The validity of the Embden-Meyerholf glycolytic pathway was made probable also in the case of the lens capsule by an investigation of Dische and Ehrlich.# These authors have shown that a stripped-off lens capsule of beef is not always able to break down glucose to lactic acid, except when ATP is added to the experimental sample. G6P, on the other hand, is rapidly metabolized. The refractory behavior of glucose is ascribed to the rapid dephosphorylation of ATP in the stripped-off lens capsule. It is well known from experiments on the metabolism of other tissues that G6P can be phosphorylated with sufficient speed at concentrations of ATP which cannot sustain an adequate rate of phosphorylation of glucose. Fluoride and bromoacetate inhibited completely the breakdown of glucose as well as of G6P and FDP. The anaerobic breakdown of ribose nucleotides in the lens capsule previously described by the authors was shown not to yield sedoheptulose phosphate and hexose-6-phosphate, as is the case in other animal tissues, and an alternative pathway for the metabolism of ribose phosphate in the lens capsule is postulated by the authors. The breakdown of G6P was shown to proceed also in the lens capsule from which the epithelium was removed. Lens capsules of rabbits break down glucose anaerobically without addition of ATP.[104] The rate of the breakdown is much higher than in cattle lens capsules supplied with ATP. The authors discuss the possible significance of the metabolism of

References 103 and 104.

the fibrous part of the lens capsule on the permeability of the lens capsule and its possible role in the pathogenesis of certain forms of cataract.

In several studies the problem of the significance of the lenticular metabolism for the permeability of the lens to water as well as to crystalloid substances has been investigated. Hauschildt and associates [105] investigated the significance of various phosphate fractions of the lens for the cation transport. They refrigerated rabbit lenses for 41 to 45 hours at 0 C in modified Tyrode's solution, which led to a shift of potassium out of the lens and its replacement by sodium. They then measured the degree of recovery after additional incubation for six hours at 37 C in individual lenses under varying conditions which affected the distribution of the phosphates of the lens between the inorganic, the seven-minutes hydrolyzable, and the total organic acid-soluble fractions. No clear-cut correlation was found between the concentration of the seven-minutes hydrolyzable and total acid-soluble phosphate and the degree of recovery, which the authors explain as due to the fact that cation distribution depends on a balance of many factors.

Ozaki,[106] on the other hand, reports that incubation in isotopic Tyrode's solution in presence of KCN or iodoacetic acid leads to a hydration of the lens which is abolished by ATP. These results are difficult to reconcile with the data of Hauschildt and associates.

Schwartz and Leinfelder [107] made an extensive study of the significance of the oxidoreduction potential on the hydration and cation equilibrium in lenses cultured in vitro. Bovine lenses which were first refrigerated for several hours were then brought to a new equilibrium by five to six hours' incubation at 37.5 C. They were then cultured for several days in the nutrient solution of Merriam and Kinsey, from which lactic acid and urea were omitted. The pH was regulated by bicarbonate and CO_2, and its variations during the whole period of the experiment were determined. The oxidoreduction potential of the nutrient solution was controlled by varying the proportions of ascorbic and hydroascorbic acid. The anaerobosis in some experiments was achieved by gassing the nutrient solution with a mixture of 95% N_2 and 5% CO_2. At various time intervals the water content of the lens, the ratio Na:K in the nutrient solution, and the potassium content of the lens were determined and correlated with the oxidoreduction potential measured in the nutrient solution. Only lens cultures to which pure hydroascorbic acid was added showed a significant weight loss, while a definite weight gain was observed under nitrogen or when pure ascorbic acid or mixtures of ascorbic acid and hydroascorbic acid were present in the nutrient solution. The hydroascorbic acid increased the potassium content of the lenses. The authors stress that their experiments indicate only certain trends as far as the influence of the oxidoreduction potential on the hydration of the lens and its cation equilibrium are concerned. Their experiments, however, suggest that the oxidoreduction potential in the aqueous and in the lens may play a significant role in the regulation of the physicochemical state of the lens.

Harris and co-workers [108] continued their study of the significance of metabolism for the permeability of the lens by studying the effect of various factors which influence metabolism on the transport of glucose into the lens. They found that under no circumstances can a higher level of glucose be obtained in the lens than is present in the surrounding medium but that, on the other hand, the rate of penetration of glucose from Tyrode's solution containing 0.4% of glucose is strongly influenced by metabolic conditions. Replacement of oxygen

by nitrogen and 0.001 M cyanide depressed significantly this rate of penetration of glucose. Still more effective was dinitrophenol at 0.01 M, fluoride at 0.05 M and iodoacetate at 0.025 M solution. The effect of fluoride could be partly reversed by 0.001 M ATP. ATP was without effect in absence of fluoride, which the authors explain by assuming that the level of ATP in normal lenses is sufficient for the normal rate of penetration of glucose. In a series of experiments the authors studied the effect of incisions in the lens capsule on the rate of penetration of glucose in presence of fluoride. They found that the incision increased the penetration of glucose and interpret it as indicating the role of the metabolism of the capsule on the penetration of glucose. As an active transport of glucose into the lens appears excluded, the authors assume that the penetration of glucose into the lens is in some way assisted by metabolic processes at the side of a barrier located in or immediately beneath the lenticular capsule.

Kleifeld and co-workers[109] arrived at similar conclusions. They investigated the rate of penetration of P^{32} into the intact lens as well as into lenses from which the anterior or the posterior capsule was removed. The experiments were carried out on lenses of calf and rabbit. Decapsulation led to increased influx of P^{32}. An increase in the permeability of the lens was also observed under the influence of metabolic poisons like KCN, NaF, and iodoacetic acid.

In further experiments Kleifeld and co-workers[110] studied the distribution of P^{32} among three different phosphate fractions in the lens which could be separated electrophoretically. The three fractions correspond to inorganic phosphate, seven-minutes hydrolyzable phosphate and the difficultly hydrolyzable phosphate. KCN in 1/1000 M solution decreased the part of P^{32} incorporated into ATP in intact lenses but not after decapsulation. NaF and iodoacetic acid interfered with the incorporation of P^{32} in ATP in intact as well as decapsulated lenses. The authors interpret these results as indicating the role of the metabolism of the capsule and the epithelium on the permeability of the lens. The significance of these results, however, appears difficult to evaluate, as the authors measured only the percentual distribution of the activity among the three phosphate fractions and did not measure in their experiments either the specific activity or the total amount of the individual phosphate fractions.

In another extensive investigation Kleifeld and co-workers[111] studied the effect on their metabolism of stimulation of the lens fibers by intermittent direct current. The experiments were based on the assumption that this type of stimulation produces changes of lens fibers comparable to those during accommodation. The lenses were stimulated in vitro by electric impulses of varying frequencies and duration for 10 to 30 minutes. Experiments in vivo were carried out on decerebrated pigeons. The lenses were stimulated by unpolarized electrodes on the limbus of the cornea. Lactic and ascorbic acid, reduced glutathione, and seven-minutes hydrolyzable P were determined on the experimental as well as on the nonstimulated contralateral lens. Decrease in the content of all these substances was observed in the stimulated lens, which, however, exceeded 10% in only one case. On the other hand, O_2 consumption was found to be increased in lenses after stimulation.

O_2 consumption was measured by a new polarographic procedure devised for this purpose.[112] Massage and treatment with ultrasonic waves of the lens did not show effects comparable to those found after electric stimulation. Nor did

continuous direct current have comparable results. The authors believe, therefore, that their findings indicate metabolic effects of a specific activity of the lens.

An important contribution to our knowledge of mechanisms involved in the formation of galactose cataract was made by Schwarz and Goldberg.[113] These authors determined the concentration of galactose-1-phosphate, the first intermediate in the metabolic breakdown of galactose, in lenses and lens capsules of normal rats as well as those of rats on galactose diet. They found that the latter at a moment when cataract developed contained several times as much of this phosphate ester as lenses of normal rats or of rats on galactose diet which did not develop cataracts. The ester was found only in the capsule and epithelium. Its minimum concentration in capsules of cataractous lenses was estimated as 170γ per gram. Galactose and glucose-1-phosphate were separated by paper chromatography with isopropyl alcohol ammonia water as solvent and were identified after hydrolysis with 0.1 N HCl for seven minutes at 100 C as galactose and glucose, respectively, by paper chromatography with *n*-butanol water as solvent and benzidine spray. The authors point out the analogy with the behavior of red cells in galactosemic children, where an accumulation of galactose-1-phosphate of the same order of magnitude as in the capsules of cataractous lenses was found associated with a decline in oxygen consumption by the red cells. The authors suggest, therefore, that the accumulation of galactose-1-phosphate interferes with the utilization of glucose, which in turn produces cataract.

Tojo[114] reports interesting observations on a synergy between galactose and xylose on the one and tyrosine on the other hand, in producing galactose cataract in scorbutic animals. Under these conditions an addition of 2% galactose or xylose together with tyrosine to the food produces cataract, while the sugars and tyrosine by themselves do not have this effect nor is a combination of glucose and tyrosine cataractogenic. The tyrosine-fed animals excreted homogentisic and benzoquinone acetic acid in the urine.

Van Heyningen and co-workers[115] determined the penetration of galactose into calf lenses in vitro and into lenses of rats on galactose diet in vivo. They observed that the calf lenses took up from a fluid containing 210 mg. galactose per 100 ml. only 30 to 50 mg. per 100 gm. lens. In cataractous lenses of galactose-fed rats the concentration of galactose went up to about two-thirds of that in the corresponding aqueous. The authors could not confirm the findings of Hörmann that feeding of cocarboxylase prevents the formation of galactose cataract.

Nordmann and Mandel[116] investigated early changes in the metabolism of the rabbit lens after irradiation. After a single dose of 1400 r (220 kv., 12 ma., 70 r per minute, 3 mm. Al filtration, 30 cm. target-skin distance, 20 mm. round field), they found two days after irradiation in the irradiated eye a significant increase in the ATPase activity as compared with that of the contralateral non-irradiated eye as control. The ATPase activity was measured by the rate of hydrolysis of ATP added to lens homogenates. The authors determined also the hexokinase activity in the irradiated eye and the controls. This was measured with fluoridated acetone powder prepared from the lens homogenate. They report a dramatic drop in the hexokinase activity in the irradiated eye after two days. The decline of hexokinase activity and the increase in the ATPase activity exceeded significantly the variations between the right and the left eyes, which the

authors found not to exceed 20%. It seems difficult to understand how an increase in the ATPase activity and a concomitant decline in hexokinase activity in vivo should not lead to a rapid deterioration of all of ATP in the lens, as the preservation of ATP in vivo can only be understood as a result of a balance between the breakdown of ATP and its resynthesis during the metabolic breakdown of glucose. It seems therefore possible that the irradiation attacks not the activity of hexokinase in vivo but only its preservation in the acetone powder.

Pirie and co-workers [117] investigated the early effects of x-ray irradiation on the content in the lens of various phosphate esters and of glutathione. They determined ATP, ADP, hexose monophosphate and diphosphate, phosphoglycerate, and inorganic phosphate as well as reduced glutathione one to several days after irradiation. The phosphate esters were determined by enzymatic methods, which were found to yield much lower values than the chemical procedures of Umbreit. No changes in the phosphate esters were found, but reduced glutathione had already declined 24 hours after irradiation.

Daisley [118] investigated the effect of x-irradiation on the ability of the lens to synthetize glutathione during a period of several months following irradiation. Glutathione synthesis was found to proceed in dialyzed homogenates of lenses of cattle and rabbit in presence of the three constituent amino acids, ATP, Mg, K, and a system reconverting ADP to ATP. As much as 70 to 140 mg. of glutathione was synthetized per hour by 100 gm. of wet lens. During the development of the radiation cataract there was a progressive loss of the ability of the lens homogenates of rabbit to synthetize glutathione, which paralleled the loss of glutathione itself. The completely opaque lens did not contain any reduced glutathione and lost completely the ability to synthetize it.

Telkkä [119] studied by histochemical methods the effect of x-ray irradiation on the SH groups of the lens as well as on the succinic dehydrogenase content. Adult rats were irradiated with 1000 r (160 kv., 4 ma., 400 r per minute, 0.5 mm. Cu 3 mm. Al filtration, 7.5 target-skin distance, 6 mm. round field); one eye was fixed in 80% ethanol with 1 to 5% TCA, paraffined, and treated according to Bennett or Barnett and Seligman for demonstration of SH groups and according to Seligman and Ruterberg for succinic acid dehydrogenase. There was in the lens a most pronounced staining in the inner cortical zone. Irradiation caused disappearance of staining in the nuclear zone, as well as in the inner cortical zone. No changes were observed in the posterior zone, and there was no succinic acid dehydrogenase in the lens, owing to the radiation effects, after several months.

Müller and his associates [120] investigated the distribution of P^{32} between three different phosphate fractions of the lens, namely, the inorganic phosphate, the 7-minute hydrolyzable P, and the difficultly hydrolyzable phosphate ester, in relation to aging and under the influence of cataractogenic agents naphthalene, dinitrophenol, and alloxan. After intravenous injection of 0.2 gm. per kilogram of alloxan there was an increase in the incorporation of P^{32} in the difficultly hydrolyzable fraction. After the injection of 10 cc. of 10% naphthalene in paraffin the radioactivity in ATP dropped by about 20%. Twenty-five per cent of dinitrophenol added to the food of 10-week-old roosters caused an acute cataract after six hours but did not induce any change in the distribution of P^{32}.

Odabara [121] reports that the development of cataract in alloxan diabetes is accelerated by protein feeding.

Orsalesi and associates [122] observed the formation of opacities in aqueous lens extracts of calf and horse after addition of extraneous electronegative and electropositive protein. The immersion [123] of whole lenses in solutions of basic proteins caused subcapsular opacities. These authors found also an effect of electropositive and electronegative proteins and of blood serum proteins on the permeability of the lens capsule of calf and horse.*

Tron and Tartakovskaja [127] also consider a relation between permeability of the lens capsule and cataract. They report that hyperparathyroidism leads to hypopermeability of the lens capsule and to cataract.

Changes in the permeability of the lens capsule can also be associated with pathological changes in other ocular tissues. Cascio and Ponte [128] find an increase in the permeability after injury of the rabbit retina by $NaIO_3$ or section of the optical nerve. They consider the possibility that nitrogenous compounds released by the injured retina effect the permeability of the lens capsule.

The nutritional requirements of the lens have been studied systematically in vitro by Kinsey and his associates.[129] They used the culture technique of Merriam and Kinsey and tested the effect of nine different media by measuring the time interval during which the mitotic rate in the lens capsule, measured according to the method of von Sallmann, remained unchanged. The media were rabbit serum and plasma ultrafiltrate, rabbit vitreous humor, beef aqueous humor, rabbit aqueous humor, and two synthetic media, TC 199 medium and KEI medium. The first of these synthetic media and rabbit aqueous humor were most efficient in preserving the rate of mitosis. In these media, however, there were signs of nuclear damage. The latter were absent in the KEI medium, which, on the other hand, preserved the rate of mitosis only to a much smaller degree. The authors explain these differences by assuming that the preservation of the lens in vitro requires certain constituents which are used up in the culture and that, on the other hand, certain toxic substances accumulate in the culture and affect the nuclei. This would explain the deleterious effect of plasma on the epithelium in these experiments. The best results were obtained with rabbit aqueous humor, which was able to preserve the normal rate of mitosis during nine hours at 37 C.

Davson [130] reports that the penetration of thiourea into the lens at constant concentration in plasma takes place during the passage of the substance from the posterior to the anterior chamber, as it is independent of the width of the pupil. It takes place more easily in young animals, and pilocarpine and atropine are without effect.

Milkman and Kennedy [88] showed that spectral sensitivity of the eyes of frogs and most fish to ultraviolet light shows a very steep rise in extinction at about 400 mμ, which in the butterfish is displaced to 360 mμ. In other fish the absorption only rises near 320 mμ. Aqueous extracts of lenses with an absorption at 400 mμ contained a dialyzable heat- and acid-stable but alkali-unstable substance which reacts with ninhydrin like an amino acid and which after oxidation shows light absorption pattern similar to that of the substance in butterfish lenses.

4. Vitreous Humor

The physical chemistry of the vitreous, particularly of its most prominent component, hyaluronic acid, has been the subject of several extensive investigations.

* References 124-126.

Woodin and Boruchoff [131] investigated the physical interrelation between the three main organic components of the vitreous, namely, the network of fibers of collagenous nature, the hyaluronic acid, and the proteins. The vitreous of ox vitreous humor was used from eyes removed 15 minutes after death. The treatment of the vitreous with trypsin, although it transformed 85% of the noncollagen nitrogen to diffusible particles, did not affect the viscosity of the vitreous. The proteins of the vitreous, therefore, do not seem to have any influence on its viscosity. The fibrous material of the vitreous could be separated from the hyaluronic acid proteins by filtration of the dialyzed and then homogenized vitreous through sintered glass filters with pores 5μ to 10μ in diameter. The fibrous material, the concentration of which was found to be 9 mg/100 gm. vitreous, was found to be combined with hyaluronic acid and to be, on the basis of the hydroxyproline-glycine ratio, closely related to other forms of collagen. The vitreous freed of fibers showed no Newtonian viscosity. Homogenates of the nondialyzed vitreous, on the other hand, which contain the whole fibrous material, showed at low shear rate an anomalous viscosity which, however, disappeared at higher shear rates. The viscosity of dialyzed vitreous from which the fibers were removed by filtration was decreased by treatment with hyaluronic acid. The viscosity was not dependent on shear. The high value of intrinsic viscosity and lack of shear dependence lead the authors to conclude that the hyaluronic acid of vitreous humor is coiled up in such a way that the over-all shape is not greatly assymmetric. This conclusion is contrary to that of other authors based on determinations of flow birefringence of hyaluronic acid. This discrepancy is attributed by the authors to the high shear rate in the latter experiments, which may have led to destruction of colloid particles. Electrophoretic studies confirm that hyaluronic acid is not combined with proteins of the vitreous. The calculation of the molecular weight from the intrinsic viscosity according to the theory of Kirkwood and Reisman and the equation of Loring and Fox leads to a molecular weight of the hyaluronic acid of vitreous humor of 500,000. The anomalous viscosity at low shear rates suggests an interaction between hyaluronic acid and the fibrous material of the vitreous which is due not to restriction by one component of the volume available to the other component but probably to coulombic forces between the positively charged fibers and the negatively charged molecules of hyaluronic acid.

The problems of the interaction of the hyaluronic acid of the vitreous and of its nonfibrous proteins was studied by Jensen and associates [132] in the vitreous of the shark Acanthias vulgaris. This investigation was based on certain physicochemical properties of hyaluronic acid which are manifested by the so-called flow elasticity and flow potential. The first can be measured by the ability of a flow-elastic liquid to give elastic recoil in a glass capillary in which it is forced to flow. This recoil is measured in millimeters. The flow potential is measured by the electropotential difference between the advancing front of the fluid column and the other front. The vitreous, obtained from eyes preserved on ice for 48 hours after death, was first treated with hyaluronidase to reduce its viscosity, and its flow elasticity was measured at different time intervals. While the recoil first increased, which is interpreted as due to the higher mobility of the fluid, it later decreased, due to the depolymerization of hyaluronic acid. An initial increase in the recoil was also observed after treatment of the native vitreous with trypsin, which was followed by a slight decrease. This indicates that the

protein component acts together with hyaluronic acid in jellifying the vitreous, and both of them must be present to form the gel. The protein also enhances the flow elasticity of the gel. The influence of the protein on the physicochemical properties of the vitreous was shown also by the fact that the flow potential of the intact vitreous had the opposite sign from that of pure hyaluronate and was reversed after trypsin treatment.

The problem of the interaction between the soluble and the fibrous residual protein of the vitreous has been approached from a somewhat different angle in an investigation by Dische and Zelmanes.[133] These authors consider the possibility that heteropolysaccharides of the type found in blood plasma as so-called serum mucoid and of those linked to plasma globulins may interact with the collagenous fibers of the vitreous and partly determine the physicochemical properties of the latter. They investigated, therefore, the composition of the polysaccharide which was found combined with the residual fibrous protein of the vitreous. The vitreous from the center of the globe was obtained almost free of pigment by thawing frozen eyes of cattle. The fibrous material, separated by high-speed centrifugation and repeated washing with distilled water, was homogenized repeatedly in a high-speed homogenizer and freed by treatment with hyaluronidase of almost all of hyaluronic acid. Of this material, 95% dissolves in 5% TCA at 90 C in 20 minutes. The determination of the carbohydrate moiety of the fibrous material showed that it essentially consisted of two hexoses, galactose and glucose, and a small amount of hexosamine, which did not exceed 10% of the total carbohydrate. The residue of the TCA extraction contained two other polysaccharides, one of which dissolved in N NaOH at room temperature and was shown by analysis to have a composition very similar to that of the carbohydrate moiety of serum mucoid and the mucoid of plasma globulins. It was also shown that the mucopolysaccharide adhered to the fibrous material only when the latter was either washed with distilled water or frozen, but not when the fibers were separated from the fresh vitreous by washing with saline. The authors concluded, therefore, that the carbohydrate moiety of the vitreous fibers is represented by a neutral heteropolysaccharide or mixture of polysaccharides with two main components, galactose and glucose, with a possible admixture of a mucopolysaccharide responsible for the hexosamine found on the fibers. In addition, a labile soluble mucoprotein in the vitreous which is very easily denatured shows a high affinity to the collagenous fibers. The authors point out the similarity between the composition of the carbohydrate of the vitreous fibers and that of the lens capsule and discuss the possibility that the type of polysaccharide found in the vitreous fibers is characteristic for collagenous fibers of the connective tissue.

Three comprehensive studies, by Laurent,[134] Varga,[135] and Brunish and coworkers,[136] deal with the molecular weight and shape of the hyaluronic acid of the vitreous. Laurent describes a new mild method of preparation of hyaluronic acid from the vitreous which consists in exhaustive dialysis of the vitreous against distilled water at 5 C and removal of cations by a resin (Dowex 50), which leads to a drop of the pH to 3.5. Under these conditions one part of the hyaluronic acid precipitates with most of the proteins of the vitreous. After centrifugation at 45,000×g and concentration of the supernatant by lyophilization, a further purification is obtained by precipitation with 6 M NaCl. The dialyzed supernatant of this precipitation contains about one-half of the originally present hyaluronic acid and a macromolecule of a molecular weight of 1.3×10^6

which can be removed by centrifugation for 24 hours at 50,000×g. The hyaluronic acid in the supernatant showed a molecular weight, as determined by light scattering, of 340,000 to 500,000. It still contained 3% protein impurities. Its intransic viscosity in 0.1 M NaCl was 2.3, from which, under the assumption of an ellipsoid shape of the molecule, a molecular weight of only 30,000 could be calculated. The molecule, therefore, has probably the shape of a random coil, and the solution in the vitreous is strongly polydisperse.

Varga [135] determined certain physicochemical constants of hyaluronic acid prepared according to the Laurent method by measuring the sedimentation and diffusion constants. The first was found to be 2.8, and the second, 2.31×10^{-7} sq. cm/second. The specific volume was thus calculated to 0.66 ml/gm. The molecular weight was calculated as 86,000. The molar friction ratio is 3.25, corresponding to a molecular asymmetry of L/d of 62, with a particle length of 900 A. and diameter of 14.5 A., on the assumption that the shape of the molecule is a rotational ellipsoid. This author also stresses the polydisperse character of the vitreous.

Brunish and co-workers [136] stress in their paper the necessity of preserving the integrity of the vitreous and the avoidance of contamination by constituents of the retina, which can be best achieved by using vitreous of eyes which are quickly frozen to −50 C before dissection. Storage at 0 C or slow freezing of the eyes were found to attack the composition of the vitreous. Sodium hyaluronate was prepared from vitreous by the authors by the detergent technique developed by N. W. Simmons, the details of which are not reported. Structural studies on this preparation were carried out by depolymerizing it with hyaluronidase and analyzing the composition of the various fractions of the degradation products. An enzyme-resistant core which remained nondialyzable was found after an exhaustive repeated treatment with the enzyme. The core, however, did not differ in its content in nitrogen and acetyl significantly from the dialyzable reaction product. The determination of the molecular weight of this preparation was carried out by light-scattering measurements and led to an estimate of the molecular weight of 400,000 to 1,000,000. The determination of the molecular length by streaming birefringence measurements led to an estimate of the molecular length of 7000 to 10,000 A. The authors conclude from this that the hyaluronic acid of the vitreous exists as an elongated unbranched molecule which is not appreciably coiled. This conclusion is completely at variance with the results of other authors. The presence of small amounts of a second, branched component of high molecular weight, however, is suggested. The authors also find evidence for the polydisperse character of the vitreous. The sharp discrepancy between the results of these authors and those of Woodin and Boruchoff and of Laurent regarding the shape of the hyaluronic acid molecule is difficult to explain. One possible reason for this discrepancy may reside in different methods of preparation of the material. It must be noted that Woodin used homogenates of the whole vitreous which contain hyaluronic acid in its natural environment, although its physiochemical properties may have been changed by the mechanical stress of the homogenization. Laurent's preparation was obtained by a very mild procedure in which the original shape of the molecule may have been better preserved, but the preparation presented less than one-half of the total hyaluronic acid of the vitreous. It is interesting that these two authors arrived at similar conclusions as far as the shape of the molecule of hyaluronic acid is concerned. Brunish and co-workers unfortunately do not give any details

of the procedure used in their preparation of hyaluronic acid. All these investigators, however, agree on the polydisperse character of the hyaluronic acid of the vitreous, stressed already some time ago by K. Meyer, and on the fact that the shape of the molecule cannot be that of a rotational ellipsoid.

Irvine and co-workers [137] also report a study of the composition of the soluble proteins of the vitreous by electrophoresis. In an untreated vitreous as well as one treated with hyaluronidase they found three different proteins, which correspond to the serum albumen and to the serum α_1- and α_2-globulins. They do not find evidence for the presence of γ-globulin, as added γ-globulin showed up after electrolysis as a new peak. This result is completely at variance with a report of other investigators on the nature of the proteins of the vitreous.

Südhof and Schweer† elaborated a new method for the determination of glucosamine in the vitreous body. This method is based on the original Elson-Morgan method. With this method they studied the content of glucosamine in the vitreous in relation to age. They found glucosamine in 2- to 3-month-old calves $99.45 \pm 4.14\gamma$/cc., in calves 8 months to 7 years old $246.64 \pm 9.43\gamma$/cc., and in calves 7 to 16 years old $168.32 \pm 7.55\gamma$/cc. The dry weight was constant up to 7 years and increased by about 10% after that. This indicates a sharp decrease in the concentration of glucosamine per unit dry weight. The significance of this finding is impossible to assess, as the authors do not distinguish between the hexosamine in hyaluronic acid and that present in the neutral mucopolysaccharides combined with proteins, the presence of which was reported by Dische and Zelmanes.[133]

The effect of hyaluronidase on the rate of diffusion in the vitreous body was investigated by Cristiansson [140] on rabbits aged 4 months to 1 year. The enzyme preparation was hyalase from H. B. Leo, Helsingfors, the activity of which is given as 200 viscosity-reducing units (VRU) per milligram. The enzyme preparation, 0.02 ml., was injected into one eye, while saline or enzyme solution completely inactivated by 36 hours' heating at 98 C was injected into the other eye. Changes in the eyes were observed for up to 35 days after the injection with a slit lamp, and depolymerization of the hyaluronic acid of the vitreous was measured at various time intervals during 17 to 34 days after the injection, by Pirie's precipitation method with acid acetone. The diffusion of labeled orthophosphate injected intraperitoneally four weeks after the injection of hyaluronidase was measured, and the specific activity in the vitreous and in the aqueous of the experimental and the control eyes was compared. While the experiments had demonstrated that the hyaluronic acid is depolymerized by the active enzyme, no change in the diffusion rate of the orthophosphate into the vitreous was induced by this depolymerization. The authors, therefore, doubt whether the structure of hyaluronic acid has any influence on the diffusion in the vitreous.

Shimek and Steffensen [141] obtained somewhat different results in their investigation of the effects of intravitreal hyaluronidase injections on the absorption of vitreous hemorrhage. One hundred fifty viscosity units of hyaluronidase injected into vitreous facilitated the absorption of injected blood without causing inflammatory symptoms in ocular tissues. The liquefaction of the vitreous was established only on the basis of inspection 15 days after injection, without precise measurements. Smaller amounts of enzyme did not cause any liquefaction, while

† References 138 and 139.

higher amounts induced severe inflammatory symptoms in the retina and uvea. The same authors also studied the effect of combined streptokinase and streptodornase injections. These injections did not lead to any liquefaction but caused severe inflammatory ocular symptoms. Injections of corticotropin (ACTH), on the other hand (7 units per kilogram per day), slowed down the absorption of injected blood significantly, which was attributed by the authors to the restraining influence of the hormone on the immigration of macrophages and on phagocytosis.

In the light of these last results, the extensive study of Dardenne and Breuer [142] on the steroid hormones of the vitreous appears of particular interest. Vitreous of cattle four hours after death was preserved under benzene at 2 C. Eight hundred grams of vitreous was extracted exhaustively with butanol at room temperature; the extract was dried down in a vacuum at 45 C, and the extract was hydrolyzed for 20 minutes with 1 N HCl and exhaustively extracted with ether. The acid fraction of steroids was taken up with 10% $NaHCO_3$, and the phenolic fraction was taken up from the ether with 1 N NaOH. The remaining fraction was then separated into the neutral ketone and the neutral nonketone fraction, and the latter was fractionated with digitonin into the α and β alcoholic fractions. The various steroid components were then identified and determined by paper chromatography, color reactions, and ultraviolet absorption curves in the neutral ketone fraction. About 0.1 mg/100 cc. of 17-ketosteroids was found with the *m*-dinitrobenzene color reaction; in the phenol fraction 1 γ/100 cc. of estrone was found by paper chromatography. In the neutral nonketone fraction the presence of an alcoholic steroid was made probable by the color reaction with antimony trichloride-benzene after separation by partition paper chromatography. In the latter fraction were found small amounts of cholesterol. The authors discuss the possible significance of the presence of these steroids for activities of various enzymes in the vitreous, particularly that of hyaluronidase.

Donn [143] achieved liquefaction of the vitreous by focused ultrasonic waves after injection of an air bubble between the vitreous and retina. The liquefaction was accompanied by the formation of a fibrous precipitate. This treatment resulted in severe damage to the retina.

Vilstrup and Kornerup [144] made a paper electrophoresis study of the proteins in the human corpus vitreum. The paper electrophoresis required previous depolymerization of hyaluronic acid by hyaluronidase and H_2O_2. They found an albumin-globulin ratio of 0.43, compared with the ratio between 0.67 and 0.74 in human plasma.

5. Retina

The biochemistry of visual pigments was significantly advanced during 1955, particularly by the experimental work of Wald, Rushton, Pitt, Morton, and their associates.

Rushton and co-workers ‡ made a fundamental contribution to the biochemistry of the retina by devising a method for the measurement of the concentration of rhodopsin in vivo. They applied this method to the eyes of albino rabbits [145] and humans.[146] The method is based on the fact that rhodopsin absorbs green light strongly but does not significantly absorb orange light. The fundus of the eye is illuminated in very rapid succession by these two kinds of lights, and

‡ References 145 and 146.

the intensity of the light reflected by the fundus is measured by a photoelectric cell, which is in the beginning set to 0 when illuminated by the two beams of light. The absorption of the green light by rhodopsin shows up in a deviation from the 0 point of the cell, which is compensated by a measured decrease in the intensity of an orange light necessary to bring the cell again to 0. Using this procedure, the authors measured the time course of bleaching of the retinas and the subsequent dark regeneration and showed that in human retinas bleaching with the light of 40,000 trolands leads after about five minutes to an equilibrium between bleaching and synthesis and a complete bleaching later can be achieved by a bright flash of light. An almost complete recovery of the rhodopsin can be then achieved after 14 minutes in darkness. They showed also the dependence of the equilibrium on the varying intensity of light. The validity of the Bunsen-Roscoe law, according to which a constant product of the intensity of the bleaching light and the time of illumination produces a constant amount of bleaching, was found in human eyes to be valid up to 48 seconds of illumination, but for longer periods the net bleaching was less than corresponded to the law. The authors found by bleaching with lights of different wave lengths that lights with equal scotopic visibility produced equal changes in the compensatory settings of the instrument, and they conclude that it is the absorption of light by rhodopsin which causes the photochemical reaction, the change of which they measured. They discuss at length the possibility that the changes in the action spectrum they measure could be produced by movements of the pigment epithelium and discard this possibility on the basis of the great speed with which these changes occur, the fact that such movements in the frog retina are stimulated by red more than by white light, and the fact that in the albino rabbit, where the measurements were made with a method which allowed direct determination of the difference spectrum of rhodopsin in the living eye, the changes in the reflected light certainly are due to the bleaching of rhodopsin. Finally, the authors determined also the topical distribution of rhodopsin in the retina of the living human eye and compared their results with the rod density distribution directly determined by Osterberg. While their measurements confirmed the absence of rods in the fovea and the optic disc and the changes in the rod distribution along a horizontal meridian agree in their sense with those found by Osterberg, no quantitative agreement between the two kinds of measurements was found. They explain these deviations by a dilution of the light coming from the rods by stray light, particularly from nerve fibers coming out from the optical nerve, which did not pass the rods. They discard on various grounds the alternative interpretation that rods near the disk have an unusually low rhodopsin content.

Weale [147] determined by a bleaching method based on the same principle as that of Rushton the absorption curve of dark- and light-adapted albino and pigmented guinea pigs. The spectra were taken with white light as well as with red, yellow, green, and blue light. The experiments suggest that four different photosensitive components are present in a guinea pig's retina. The paper describes in detail the experimental procedure.

Pitt and co-workers [148] made an attempt to elucidate the nature of the bond between retinene and opsine by determining the nature of the bond between this aldehyde and protein in the indicator yellow which appears as a transient intermediate during the bleaching of rhodopsin. Starting from the proposition that retinene forms Schiff's base linkages with free amino groups of the protein, they

prepared a Schiff's base from retinene and methylamine, which they call retinylidenemethylamine. This compound was an indicator and its Schiff's base structure was inferred from the near identity of its absorption curve with those obtained from retinene oxime, as far as the direct spectrum of the compound itself is concerned. The spectra in the antimony trichloride color reaction of the two compounds differ somewhat in the position and intensity of the maximum, but they shared four different features with each other which were not found in other vitamin A derivatives. Retinylidenemethylamine is extremely unstable and is readily hydrolyzed by ethanol or water over a wide pH range. This hydrolysis, however, can be suppressed by an excess of methylamine. It is also easily attacked by oxygen. Retinylidenemethylamine is converted by dry HCl in light petroleum solution to a product which dissolved in chloroform gives a characteristic absorption spectrum, which by analogy with the behavior of synthesized apocarotenylidenemethylamine is interpreted as a substituted ammonium salt of retinylidenemethylamine. This structure is also supported by the analogous optical properties of a synthetic retinylidenedimethylammonium iodide. Retinylidenemethylamine also forms chromogens in concentrated sulphuric acid with a fading peak at 515 to 520mμ and a stable peak at 430 to 435mμ. The authors point out that the optical properties of the chromogens formed from retinylidenemethylamine by H_2SO_4 resemble in some respects those of rhodopsin and, although they are not photosensitive and are produced under grossly unphysiological conditions, they bring out potentialities of retinylideneamino compounds which deserve consideration in investigations on the structure of rhodopsin.

Morton and Pitt [149] studied further the analogy between retinylidenemethylamine and rhodopsin, by determining the stability of the synthetic analogue over a wide range of pH and comparing it with that of indicator yellow. The retinylidenedimethylammonium chloride present in solution at pH 1.0 was completely stable for a period of 18 hours, after which time a fall in extinction of the curve due to nonhydrolytic destruction took place. At higher pH the analogue was less stable, with a maximum of hydrolysis rate at pH 6.6. At a still higher pH the hydrolysis was slowed down again. The stability of the acid indicator yellow of Lythgoe is interpreted as analogous to the stability of the retinylidenemethylamine at pH 1.0. The higher stability of the indicator yellow in alkaline solutions is explained by the presence of an excess of amino groups of proteins in solutions in which the bleaching of the visual pigments was studied. This concept appears to be borne out by the observation of Collins (1953) that the indicator yellow is rapidly hydrolyzed in alkaline solutions if all free amino groups of opsine are blocked by formaldehyde. An analogous result could be obtained by adding formaldehyde to the solution of the synthetic analogue, under which condition the hydrolysis of the latter was accelerated, while excess of methylamine inhibited hydrolysis. The authors point out that the indicator yellow formed from rhodopsin solutions in the dark at pH 4.0 should be regarded as a direct derivative of rhodopsin, provided that it has not been passed at any time through alkaline solutions containing an excess of unionized amino groups, in which case it is probably a mixture of various unphysiological retinylideneamines. They believe, therefore, that the study of the indicator yellow under acid conditions opens an experimental possibility for the identification of the amino group to which retinene is linked with rhodopsin.

Wald and co-workers [150] made an exhaustive study of the chemical structure of neoretinene b, the carotenoid moiety of the two visual pigments. After having shown that neoretinene b must be a hindered cis isomer of retinene, they prepared by synthesis a hindered cis isomer which they regarded as 11-cis isomer and which proved not to be identical with neoretinene b and was designated as neoretinene c. From that they concluded that neoretinene b must be the 7-cis isomer, which so far has not been prepared synthetically. In a preliminary communication later, however, the authors corrected these statements after having established that neoretinene c is identical with 11,13-dicis retinene and that the synthetically prepared 11-cis isomer seems to be identical with neoretinene b.

Hubbard [151] found an enzyme in cattle retinas which catalyzes specifically the interconversion between all-trans retinene and neoretinene b. The enzyme shows a strong acceleration of its activity in dim light. The action of the enzyme finally leads to a pseudoequilibrium mixture containing 35% to 40% neoretinene b. The enzyme, therefore, is a photoisomerase. Light promotes the reaction in the direction of neoretinene b but has little effect on the reverse reaction. The presence of this enzyme in the retinas appears of fundamental importance as the reaction product of the bleaching of the visual pigments, the all-trans retinene, is unable to recombine with the opsines to become rhodopsin.

Wald and co-workers [152] reported results of their extensive investigations on the nature of iodopsin. They clearly demonstrated that the carotenoid moiety of iodopsin is identical with that of rhodopsin, the difference between the two pigments residing with the protein to which the chromophoric group is attached. Chicken retinas, which contain many more cones than rods, were used as a source of pigments. In a first series of experiments the identification of the product of bleaching iodopsin was carried out on dried adult chicken retinas dried by lyophilization, from which the main bulk of carotenoids was removed by petroleum ether extraction. The residue was extracted three times with hexane in the dark, in red light, and in bright white light, respectively. The dark extract, containing only remaining carotenoid not extracted by petroleum ether, was used to evaluate the contributions of small amounts of carotenoid present in the two other extracts. The products of bleaching in the two illuminated extracts were then determined by the light-absorption curve, which after correction for the carotenroid showed an identical shape for both extracts. To complete the identification the three hexane extracts were transferred to chloroform and the absorption curves of the reaction product with antimony trichloride were determined. In a second series of experiments it was shown that the carotenoid moiety of iodopsin is reduced by the intact retina to vitamin A_1. Of two sets of dark-adapted chicken retinas washed with saline in the dark, one was dried and extracted with petroleum ether immediately and the other, after bleaching with red light. The two petroleum ether extracts were chromatogramed on a calcium carbonate column, and with the fraction corresponding to vitamin A an antimony trichloride test was carried out, which in the bleached retinas showed the spectrum characteristic for vitamin A, while the nonbleached dried retinas showed predominantly retinene and only small amounts of vitamin A preformed in vivo. In a third series of experiments the bleaching of iodopsin and rhodopsin was carried out with solutions of these pigments obtained by extraction in the dark with a 0.2% digitonin solution of outer segments of rods and cones of chicken retinas separated by fractionated centrifugation, the details of which are reported, and dried and extracted with

petroleum ether previously to the digitonin treatment. These solutions were found to contain one and one-half times as much rhodopsin as iodopsin. By bleaching these solutions first with red and then with white light and determining the absorption curves before and after the bleaching, different spectra were obtained for iodopsin and rhodopsin, the first showing a maximum at 562mμ, the second, at 509mμ. The spectra of the two retinenes obtained in this way differed slightly inasmuch as the spectrum of the retinene from iodopsin showed a displacement of its maximum of 5 to 6mμ toward shorter wave lengths, which is to be expected as a result of the isomerization of all the trans isomer of retinene formed by the white light used to bleach rhodopsin. The synthesis of iodopsin finally was achieved by adding the cis-trans isomer of retinene, neoretinene b, to a cone opsine solution obtained by red-light bleaching of a chicken retinal extract. The synthesis was completed after about 10 minutes, and it was possible to separate completely the synthesis of iodopsin from that of rhodopsin by adding first the amount of neoretinene b sufficient only for the synthesis of the first pigment and subsequently that necessary for the synthesis of rhodopsin, which required much more time for completion. Kinetic experiments showed that the bleaching of iodopsin is a first-order, its synthesis a second-order, bimolecular reaction. The rate of iodopsin synthesis was found to be 527 times that of rhodopsin synthesis. Iodopsin was found to be much less stable against changes of pH than rhodopsin. While there was little fall of extinction between pH 5 and 7, it fell rapidly on either side of this range and disappeared completely after one hour at pH 3.7 and 9. The poisons which inhibit the synthesis of rhodopsin by combining with either retinene or opsine were found in general not only to interfere with the synthesis of iodopsin but to bleach this pigment. *p*-Chloromercuribenzoate bleached above 3×10^{-5} M, while for complete blocking of synthesis 1.8×10^{-4} M was required, that is, much more than for blocking rhodopsin synthesis. The bleaching effect was not reversed by excess SH groups, which indicates that the bleaching is due to deep-seated changes in the molecule comparable with denaturation. The hydroxylamine effect on iodopsin differs also from that on rhodopsin in that it destroys the first pigment in concentrations which do not prevent its synthesis. The authors used the data obtained in these experiments to determine by two different procedures the absorption spectrum of iodopsin. They found the following characteristics: an α band maximum at 562mμ, a minimum at about 435mμ of an extinction 0.2 times that at 562mμ, and a small β band at about 370mμ of extinction 0.32 to 0.35 times that at 562mμ. They also found that per unit weight of retinene iodopsin and rhodopsin have nearly the same extinction. The paper finally discusses, on the basis of the experimental results, the correlations between (*a*) pigment concentration and visual sensitivity, which permit only the conclusion that the ratio of sensitivities of rod and cone in the intact animal is of the same order as the ratio of absorptions of visual pigments in the rods and cones; (*b*) the relation of dark adaptation and synthesis of visual pigments based on the compartmentation hypothesis of the receptor structure of Wald and co-workers (they find a striking parallelism between the course of dark adaptation of rod and cone vision and the synthesis of visual pigment and that the connection between these functions relates the concentration of visual pigment to the logarithm of the visual sensitivity); (*c*) the relation between the absorption spectra of the two visual pigments and the spectral sensitivity, which for iodopsin, even after correction for the absorption due to the transparent media of the eye,

shows a displacement of the photopic sensitivity of about 20mμ towards the blue from iodopsin. This deviation in humans is explained by the trichromatic character of the cone pigments in humans, which, according to Stiles' analysis, possess maxima at about 440, 550, and 590mμ.

The experiments of Weale,[153] carried out on four dark-adapted gray squirrels which were anesthesized and currarized, showed a bleaching spectrum (corrected for light scattered by the lens) with a maximum of 535mμ. The curve differed also in its shape from that found by the same methods in guinea pigs, rabbits, and cats in previous investigations.

Arden and Tansley [154] also determined the spectral sensitivity of the retina of the gray squirrel (Sciurus carolinensis leucotis) by an electroretinographic method. The paper contains details about the histological structure and the form of the electroretinogram of the animal. The latter support the conclusion that the retina of the gray squirrel contains only cones, which could not be established completely on purely histological grounds. Consequently, in the response to light and dark adaptation the squirrel eye behaves as if it contained only cones, and there was no sign found of a Purkinje shift. The sensitivity curve was obtained by exposing the animals to stimuli of different wave lengths first selected at random and then in an orderly fashion from the shortest to the longest waves and back. It was possible to detect in this way any changes of sensitivity during the experiment. The sensitivity curve had a maximum around 520mμ, and it was completely different from the absorption curves of iodopsin and rhodopsin and most nearly resembled the sensitivity curve found by Granit for the green modulator of the cat. The authors point out the agreement between their results and those of Weale obtained on the gray squirrel by the in vivo bleaching method and suggest that their sensitivity curve is determined by photochemical reactions in the retina.

Arden and Tansley [155] also determined the spectral sensitivity curve of the pure-cone retina of the European ground squirrel, or suslik (Citellus citellus), which is also a diurnal animal and on histological grounds is assumed to have eyes containing only cones. They give a detailed distribution of the histological structure of this retina. The sensitivity curve was also measured by electroretinography. The experiments showed that the retina of the suslik resembles completely, in the histological picture and in the shape of the electroretinogram, that of the gray squirrel. Correspondingly, they did not find any Purkinje shift in the suslik and there was a maximum spectrum sensitivity at about the same wave lengths, between 520 and 530mμ. In some experiments, however, the spectral sensitivity curve indicated the presence of a second maximum, perhaps near 470mμ. This response, however, to blue light could not be obtained in all experiments, a variability of results which the authors are unable to explain.

Wald and Burg [156] identified the vitamin A which is stored in the lobster eye as the cis isomer, neovitamin Ab, the specific precursor of rhodopsin. The identification was carried out by the determination of the absorption spectrum of the vitamin as well as by its oxidation with MnO_2 to an aldehyde which was demonstrated to be identical with neoretinene b. Neovitamin Ab was found to possess only 23% of the nutritional potency of all the vitamin A in rats.

Lambertsen [157] found also a new, not identified, vitamin A isomer in eyes of Pandalus borealis.

Vitamin A was found to be present in the retina as an ester of fatty acids. Krinsky [158] succeeded in obtaining cell-free retinal preparations which are able to esterify added all trans vitamin A. The enzyme was prepared from bleached lyophilized and pulverized cattle retinas which were extracted with petroleum ether. When the enzyme material was suspended in a phosphate buffer of pH 7.7, 70% of the added vitamin was found esterified after one hour at 37 C. The preparations also hydrolyzed vitamin A esters, the acetate more rapidly than palmitate. The particulate matter in the preparation was only active after addition of the supernatant, and the latter could not be replaced by triglycerides, lecithin, or fatty acids.

Noell [159] studied the histological changes produced in the receptor cells of the retina of albino rabbits by high oxygen pressure and compared them with the injury previously described from intravenous administration of iodate, iodoacetate, and x-rays. He also carried out a few experiments on the effect of iodoacetate, x-irradiation, and high oxygen tension on the glycolysis and respiration on the retina in vitro. Albino rats of 2 to 3 kg. body weight were exposed to oxygen pressures ranging from 350 mm. Hg upwards at ambient pressure and up to 90 lb/sq. cm. The injury to the retinal elements was studied at time intervals ranging between 10 hours and 16 days, and the exposure to oxygen was up to 40 hours. Ten to twenty-four hours after exposure to 100% O_2 the sensory organelles, as well as the outer nuclear layer, had already deteriorated. Some of the nuclei in the nuclear layer were autolyzed; others were pyknotic. Sixteen days after exposure to 100% oxygen for 40 hours the injury had progressed and the majority of visual cells had disappeared. The outer nuclear layer was reduced to one or two rows of nuclei. Of the receptor cells, only some stumps of inner limbs remained. There was a characteristic pattern of topical distribution of the injury over the retina which was practically the same as after poisoning with iodoacetate. It was not, however, possible to destroy all visual cells by exposure to high-tension oxygen. The remaining ones had a cone-like appearance. Poisoning with iodoacetate and exposure to x-rays lead to more complete disappearance of visual cells. The author concludes that cone cells are more resistant to oxygen than rod cells. The surviving cells, however, showed disformed sensory organelles. This disformation is explained as a secondary effect due either to mechanical stress on the surviving cones caused by the degeneration of rod cells or as the expression of a disturbance of retinal homostasis similar to that produced by iodate. This second concept is supported by the injurious effect of high oxygen tension on the uveal tissues. Metabolic studies on retinas exposed to oxygen, x-rays, and iodoacetate did not show an identical pattern of changes. Iodoacetate (20 mg. per kilogram intraveneously), as expected, caused a sharp decrease in anaerobic and aerobic glycolysis and a lesser decrease in oxygen consumption. X-irradiation affected only the respiration and not the glycolysis, while oxygen decreased glycolysis by no more than one-third and did not affect oxygen uptake significantly. The injury to the visual cells, therefore, cannot at present be correlated with clear-cut metabolic effects.

Francis [160] carried out an extensive histochemical study on the distribution of various forms of lipids in different segments of the retina. He determined liquid lipids by their staining with Sudan dyes, neutral lipids by staining with Nile blue, and saturated ones by staining with osmium tetroxide. Phospholipids were determined by the acid hematin method with pyridine extraction as control

(Zaker) and lecithin, by the method of Romieu; mitochondria, by the method of Kane; cholesterol, by Schultz's modification of the Liebermann-Burchardt reaction; carotenoids, by staining with methyl green-pyronin and the Carr-Price (antimony trichloride) reaction. Finally, lipid aldehydes were determined by the method of Hayes and of Lilly, based on the oxidation of these substances with periodic acid, chromic acid, or potassium permanganate. The results of these histochemical determinations do not appear very informative and are mostly not in agreement with direct chemical determinations. Thus, the reaction for phospholipids was weak in the outer segments of rods and cones, although according to Collins (1950) the direct analysis shows 31.9% of phospholipids in these segments. This discrepancy is explained by the possibility that these phospholipids are partly eluted during the dying procedure. The same situation prevailed with carotenoids, which showed only a patchy distribution in outer limbs with the histochemical method but showed up consistently in the elipsoids. The author explains the failure to detect any carotenoid at all in the outer segments with the Carr-Price reaction by the possibility that the carotenoid combined with proteins does not react. The oil droplets in the inner segments of the chick and pigeon eyes stained with Sudan dyes and osmium tetroxide, but the colorless ones of the frog were dyed only by the latter. The author mentioned the possibility of unsaturated fats in solid state as basis of the colorless droplets to explain this difference. The antimony trichloride test carried out with the reagent liquefied on standing without the use of any organic solvent, showed a rapidly fading blue color in all droplets in the pigment epithelium and the chorioid and a more stable one in the oil droplets of the retina. This is related to the possible presence of vitamin A in the first two tissues and other carotenoids in the oil droplets of the retina. The ellipsoids gave a positive color in the acid-fuchsin staining for mitochondria and for phospholipids in general and lecithin in particular. This is in agreement with the findings of other authors about localization of mitochondria in the sensory organelles of the retina. In the synaptic layers of the retina considerable amounts of cholesterol were found by the author, who discusses the possibility that its presence in the synapses may be related to the transmission of nerve impulses if mechanisms of the latter suggested by Beutner and Barns were valid.

Gourewitch [161] investigated the controversial problem of whether illumination of the retina leads to hydrolysis of organic phosphate esters. Four retinas of frog or two retinas of chicken were incubated after dark adaptation for several periods of 30 minutes each in Ringer solution at room temperature; the suspension fluid was separated by filtration, and the inorganic phosphate was determined in it by the Kuttner method. No significant amounts of inorganic phosphate were found in any suspension fluid when the incubation of the retina was carried out in bright light. The experimental procedure of the author appears objectionable, as it is based on the assumption that all inorganic phosphate which was split off would diffuse into the suspension fluid. The method of determination of inorganic phosphate, apparently without preceeding deproteinization, appears of doubtful value.

Hebb [162] determined the activity of choline-acetylase in the retina, the optic nerve, the sensory roots, and the cerebral cortex of birds and mammals by a method which eliminated errors due to variations in the rate of coenzyme A (CoA) acetylation. The retina of chicken and pigeon was found to produce more acetylcholine per unit weight of tissue in a given time than any other vertebrate tissue yet

examined, including the optic lobes, which contain high concentrations of the enzyme. On the other hand, no significant amounts of this enzyme were found in the optic nerve of the chicken, and small amounts were found in the optic nerve of the pigeon. The author tries to explain the latter fact by the possible presence of some cholinergic nerves in the optic nerve of the pigeon. The author interprets these results, as well as the lack of demonstrable choline-acetylase in the sensory roots of the bullock, as a sign that this enzyme plays no role in the conductivity of peripheral nerves. The possibility that the differences in the enzymatic activity between sensory and other nerves may be due rather to extractibility, accessibility, and variations in its stability cannot be excluded on the basis of these experiments.

Coulombre [163] studied correlations between processes of the differentiation and the activity of the enzyme hydrolyzing adenosine triphosphate (ATPase) (apyrase) in the retina of the chick. The enzyme was found to increase with the onset of certain phases of differentiation, namely, on the 8th day with the development of the inner and on the 10th day the development of the outer plexiform layer, the stoppage of mitosis in the pigment epithelium, appearance of the inner segments of rods and cones, and reduction of the ganglion cells to one layer. Another increase associated with an increase in alkaline phosphatase was found on the 15th day, characterized by the development of outer layers of rods, of oil droplets in cones, of astaxanthin, and by cessation of ganglion-cell divisions. Two types of adenosine triphosphatase were found, one in the pigment epithelium, activated by Mg and Ca, and one in the neutral retina, activated only by Mg. The activity of the enzyme was measured in homogenates diluted 25-fold with aminoacetic acid buffer of pH 8.95.

Rosen and co-workers [164] found that while diabetics excreted larger quantities of xanthuronic acid after an oral load of 10 gm. DL-tryptophan than normal controls there was no difference between patients with and those without retinopathy. The excretion could be reduced by intramuscular pyridoxine injections. Vitamin B_6 deficiency induced by 100 mg. of deoxypyridoxine increases excretion of xanthuronic acid but does not effect retinopathy.

Maengwyn-Davis and co-workers [165] compared the excretion of acetylated sulfadiazine 24 hours after administration of the drug to diabetics with and without retinopathy and to controls. While the over-all excretion of the acetylated drug was slightly but significantly greater in diabetics, there was no difference between those with and those without retinopathy.

Hayano [166] reported that light adaptation produces a decrease of the pH of the retina, which he relates to changes in the visual purple.

Koride [167] determined the optimum pH of the alcohol dehydrogenase of the retina to be 8.1. The enzymatic activity was increased by an extract of the chorioid, and this effect was accentuated by ATP, Mg, glutathione (GSH), and succinate.

Kanapa [168] reports that digitonin extracts of the shrimp Euphasia pacifica contain a photosensitive pigment with maximum decrease of optical density by bleaching at 462mμ and vitamin A, but not retinene, as product of bleaching.

Matsuralia [169] reports that the b and c waves of the chick retina disappear after iodoacetic acid (IAA). IAA also induces a change in the polysaccharide of inner

segments of cones and disappearance of miscellaneous ribonucleic acid in the ellipsoid.

Van Den Hoof [3] made an electron microscopic study of the sclera and found densely intertwined collagen fibers with diameters varying between 400 and 1400 A. These collagen fibers were characterized by the usual periodicity of 640 A. In the tapetum fibrosum collagen fibers were found with the usual periodicity of 640 A, but the fibers were very much shorter than in other eye tissues, the shortest being only about 50μ long. This the author related to the pulp-like consistency of this tissue. Bruch's membrane was investigated by a special technique of layer stripping, and it was found that the part facing the pigment epithelium is filled with granula and numerous openings of various widths. In the fibrous part of the membrane two spatially separated fibrous components were found, one of which is represented by collagen and the other is presumed to be elastin.

Smelser and Ozanics [170] continued their investigations of the exophthalmos-producing substances present in pituitary extracts. They made an attempt to determine the nature of the substance in the pituitary extract which in presence of a potentiating factor in corticotropin produces exophthalmos, as well as the nature of the potentiating factor itself. The experiments were carried out on young female albino guinea pigs (280 to 300 gm.). The degree of exophthalmos was measured by the distance from the skull at the supraorbital notch to the limbus. The measurements were carried out after death, and the fat, muscle, and glands of the orbit were weighed and the degree of edematous infiltration of the fat was noted. Five units of corticotropin in gelatin (Armour) injected simultaneously with 5 mg. of a standard beef pituitary extract daily into 12 thyroidectomized guinea pigs produced a good exophthalmos, although the two extracts separately had no effect. A highly concentrated corticotropin extract, 50γ to 100γ, could replace the original corticotropin preparation. A potentiating effect was also found with 2.5 mg. of cortisone in a group of 14 guinea pigs. The authors conclude, therefore, that the potentiating agent in corticotropin preparations is corticotropin itself and that its effect is due to an increased production of some adrenal steroids. The authors then made an attempt to determine the nature of the compound in the pituitary beef extract which is exophthalmogenic in presence of corticotropin or cortisone. Prolactin and two different preparations, each of growth hormone and gonadotropins in quantities 16 times as great as that present in the 5 mg. of the standard pituitary preparation (which was exophthalmogenic), were injected together with cortisone, and they did not produce in any case exophthalmos or unequivocal changes in the fat or Harderian gland. This shows that the exophthalmogenic factor in the standard pituitary extract is not identical with any of these three hormones. These hormone preparations contained only traces of thyroid-stimulating hormone. On the other hand, daily injections of only 2 units of thyroid-stimulating hormone plus 5 to 12.5 mg. of cortisone into two guinea pigs produced slight, but unmistakable, changes in the orbital fat and protrusion of the globe. On the other hand, a pituitary extract from horse which contained very little thyrotropic hormone was extremely effective when injected with cortisone. An injection of a combination of growth hormone, prolactin, gonadotrophin, corticotropin, and cortisone which contained less than 2 units of thyroid-stimulating hormone had a very intensive exophthalmogenic effect, which exceeded almost four times that which would account for the amount of thyro-

trophic hormone present in the mixture. The authors conclude, therefore, that the pituitary, in addition to the thyrotropic hormone, contains still another constituent, with a much greater exophthalmogenic effect.

Weitrel and co-workers [171] found that tapetal tissue of dog and fox contains a zinc sulfur compound which on the basis of extraction with acid, paper chromatography, and SH group titration with I_2 is assumed to be a Zn-cysteine complex.

Baron and co-workers [172] compared the Na/ Kratio in the eyes of mice in darkness and in light, respectively, and in eyes of 40 men, 20 normal and 20 with glaucoma. While there was no difference between dark- and light-adapted eyes of men, there was a significant decrease in the ratio in illuminated mice. At the same time, in these animals there was an increase of about 0.1 in the pH of the eye tissues.

Terner and co-workers [173] reviewed the literature on the radioactive phosphorus (P^{32})-uptake test in 262 cases of ocular and adnexal pathology.

630 West 168th St. (32).

REFERENCES

1. François, J., and Rabaey, M.: Proteins of Corneal Epithelium, Bull. Soc. belge opht. 108:641, 1955.

2. Münich, W., and Oswald, A.: Paper Electrophoresis Studies on Various Water-Soluble Organ Proteins of the Calf's Eye, von Graefes Arch. Ophth. 156:547, 1955.

3. van den Hooff: Electron Microscopic Studies of Some Ophthalmic Fibrous Structures, Ophthalmologica 129:60, 1955.

4. Dohlman, C. H., and Balázs, E. A.: Chemical Studies on Descemet's Membrane of the Bovine Cornea, Arch. Biochem. 57:445, 1955.

5. Dohlman, C. H., and Boström, H.: Uptake of Sulfate by Mucopolysaccharides in the rat Cornea and Sclera, Acta ophth. 33:455, 1955.

6. Schiff, F. S.: Cystine Deposits of Cornea and Conjunctiva, A. M. A. Arch. Ophth. 53:434, 1955.

7. Cagianut, B.: Research on the Substrate of the Kayser-Fleischer Corneal Rings, Ophthalmologica 129:218, 1955.

8. Kinoshita, J. H.; Masurat, T., and Helfant, M.: Pathways of Glucose Metabolism in Corneal Epithelium, Science 122:72, 1955.

9. Cogan, D. G., and Kuwabara, T.: Lipogenesis by Cells of Cornea, A. M. A. Arch. Path. 59:453, 1955.

10. Cogan, D. G., and Kuwabara, T.: Fat Formation and Foam Cells Following Injection of Brain Tissue into Cornea, J. Neuropath. & Exper. Neurol. 14:95, 1955.

11. Gloster, J.: Investigation of the Carbonic Anhydrase Content of the Cornea of the Rabbit, Brit. J. Ophth. 39:743, 1955.

12. Iida, M.: Phosphatase of the Cornea, Acta Soc. Ophth. japon. 59:44; 109, 1955.

13. Davson, H.: The Hydration of the Cornea, Biochem. J. 59:24, 1955.

14. Langham, M. E.: The Effect of Aerobic Metabolism on the Movement of Water and Sodium in the Rabbit Cornea, J. Physiol. 128:43P, 1955.

15. Harris, J. E., and Nordquist, L. T.: The Hydration of the Cornea: I. The Transport of Water from the Cornea, Am. J. Ophth. 40:100 (Nov., Pt. II) 1955.

16. Philpot, F. J.: Factors Affecting the Hydration of the Rabbit Cornea, J. Physiol. 128:504, 1955.

17. Smelser, G. K., and Chen, D. K.: Physiological Changes in Cornea Induced by Contact Lenses, A. M. A. Arch. Ophth. 53:676, 1955.

18. Smelser, G. K., and Chen, D. K.: A Comparative Study of the Structure and Hydration Properties of Corneas Adapted to Air and Aquatic Environments, Acta XVII Conc. Ophth. (1954) 1:44, 1955.

19. Maurice, D. M.: Influence on Corneal Permeability of Bathing with Solutions of Differing Reaction and Tonicity, Brit. J. Ophth. 39:463, 1955.

20. François, J., and Rabaey, M.: New Contribution to Physico- and Histochemical Studies of the Cornea, Acta XVII Conc. Ophth. (1954) 3:1739, 1955.

21. Grant, W. M., and Kern, H. L.: Action of Alkalies on the Corneal Stroma, A. M. A. Arch. Ophth. 54:931, 1955.

22. Guidry, M. A.; Allen, J. H., and Kelly, J. B.: Some Biochemical Characteristics of Acid Injury of the Cornea: I. Ascorbic Acid Studies, Am. J. Ophth. 40:111 (Nov. Pt. II) 1955.

23. De Simone, S.: Oxygen Consumption and Anaerobic Glycolysis of the Rabbit Cornea in Experimental Neuroparalytic Keratitis, Boll. ocul. 34:129, 1955.

24. Sakai, M.: Ion Concentration of Living Corneas: III-IV, Acta Soc. ophth. japon. 59:632, 1955.

25. Wiesinger, H.; Kaunitz, H., and Slanetz, C. A.: Corneal Changes in Riboflavin-Deficient Rats, Ophthalmologica 129:389, 1955.

26. Rokhlina, M. L., and Zubarova, T. V.: Effect of Pantothenic Acid and of Vitamin B_6 on Regeneration of the Corneal Epithelium, Bull. Exper. Biol. & Med., (Russian) 40:58, 1955.

27. Gandolfi, A., and Montaldi, M.: The Possibility of Eliminating the Antiepithelializing Effect of Hydrocortisone by the Use of Chlorophyll, Arch. Ottol. 59:5, 1955.

28. Gezurian, Z. L., and Persoglia, J. M.: Effects of Cortisone Acetate Instillation on the Corneal Epithelium of the Guinea Pig, Semana méd. 107:544, 1955.

29. Passmore, J. W., and King, J. H., Jr.: Vital Staining of Conjunctiva and Cornea: Review of Literature and Critical Study of Certain Dyes, A. M. A. Arch. Ophth. 53:568, 1955.

30. Lewke, J.: Pathways of Metabolic Exchange in the Cornea, Anat. Anz. 101:237, 1955.

31. Wislocki, G. B., and Ladman, A. Y.: The Demonstration of a Blood-Ocular Barrier in the Albino Rat by Means of the Intravitam Deposition of Silver, J. Biophys. & Biochem. Cytol. 1:501, 1955.

32. Rao, S. S.; Kulkarni, M. E.; Cooper, S. N., and Radnakrishnan, M. R.: Analysis of Proteins of Bovine Lens, Vitreous, and Aqueous by Electrophoresis and by Oudin's Gel Diffusion Technique, Brit. J. Ophth. 39:163, 1955.

33. Keup, W., and Steiger, R.: Amino Acid Elements of Normal Human Aqueous Fluid Protein, Brit. J. Ophth. 39:503, 1955.

34. Davson, H.: A Comparative Study of the Aqueous Humour and Cerebrospinal Fluid in the Rabbit, J. Physiol. 129:111, 1955.

35. Davson, H.: Adsorption of Thiocyanate to the Plasma Proteins: With Special Reference to the Distribution of This Ion Between Aqueous Humour and Plasma, Brit. J. Ophth. 39:681, 1955.

36. Friedenwald, J. S., and Becker, B.: Aqueous Humor Dynamics: Theoretical Considerations, A. M. A. Arch. Ophth. 54:799, 1955.

37. Lee, P. M.: The Effect of a Carbonic Anhydrase Inhibition on the pH, Bicarbonate, and CO_2 Concentrations in Blood and Aqueous Humour, J. Physiol. 128:80P, 1955.

38. Gloster, J.: The Effect of a Carbonic Anhydrase Inhibitor on the Intraocular Pressure in Rabbits, J. Physiol. 128:77P, 1955.

39. Davson, H., and Luck, C. P.: The Distribution of Bicarbonate between Aqueous Humour, Cerebrospinal Fluid and Plasma in Several Mammalian Species, J. Physiol. 130:48P, 1955.

40. Langham, M. E.: Effect of Carbonic Anhydrase Inhibitor on the Circulation of the Aqueous Humour, J. Physiol. 128:78P, 1955.

41. Becker, B., and Constant, M. A.: Experimental Tonography: The Effect of the Carbonic Anhydrase Inhibitor Acetazoleamide on Aqueous Flow, A. M. A. Arch. Ophth. 54:321, 1955.

42. Becker, B.: The Effects of the Carbonic Anhydrase Inhibitor, Acetazoleamide, on the Composition of the Aqueous Humor, Am. J. Ophth. 40:129 (Nov., Pt. II) 1955.

43. Kinsey, V. E.; Camacho, E.; Cavanaugh, G. A., and Constant, M. A.: Diamox and Intraocular Fluid Dynamics, Am. J. Ophth. 40:147 (Nov., Pt. II) 1955.

44. Appelmans, M. J.: Aqueous Humor of the Rabbit Following Administration of Diamox, Bull. Soc. belge ophth. 108:521, 1955.

45. Falbriand, A.; Zender, R.; Sauz, M. C., and Franceschetti, A.: The Potassium and Sodium of the Aqueous Humor of the Rabbit and Their Variations Under the Influence of Catazoleamide, Experientia 11:232, 1955.

46. Falbriand, A.; Sauz, M. C.; Zender, R., and Franceschetti, A.: Action of Carbonic Anhydrase Inhibitor, Diamox, on the pH of Aqueous Humor, Experientia 11:385, 1955.

47. Sugar, S., and Fainstein, S.: The Coefficient of Outflow of Aqueous: With the Water Test and Following the Use of Diamox, Am. J. Ophth. 40:693 (Nov., Pt. I) 1955.

48. Weinstein, P., and Forgáes, J.: Data Concerning the Mechanism of the Eye Tension Lowering Effect of Diamox, Ophthalmologica 130:159, 1955.

49. Green, H.; Bocher, C. A.; Calnan, A. F., and Leopold, I. H.: Carbonic Anhydrase and the Maintenance of Intraocular Tension, A. M. A. Arch. Ophth. 53:463, 1955.

50. Green, H.; Bocher, C. A., and Leopold, I. H.: Carbonic Anhydrase and the Elaboration of Bicarbonate Ion in the Rabbit Eye, A. M. A. Arch. Ophth. 53:472, 1955.

51. Friedenwald, J. S.: Carbonic Anhydrase Inhibition and Aqueous Flow, Am. J. Ophth. 39:59 (April, Pt. II) 1955.

52. Friedenwald, J. S.: Current Studies on Acetazoleamide (Diamox) and Aqueous Humor Flow, Am. J. Ophth. 40:139 (Nov., Pt. II) 1955.

53. Green, H.; Sawyer, J. L., and Leopold, I. H.: Determination of Acetazoleamide in Aqueous Humor of the Rabbit Eye, A. M. A. Arch. Ophth. 53:478, 1955.

54. Ballintine, E. J., and Maren, T. H.: Carbonic Anhydrase Activity and the Distribution of Diamox in the Rabbit Eye, Am. J. Ophth. 40:148 (Nov., Pt. II) 1955.

55. Kinsey, V. E.; Camacho, E.; Cavanaugh, G. A.; Constant, M., and McGinty, D. A.: Dependence of IOP-Lowering Effect of Acetazoleamide on Salt, A. M. A. Arch. Ophth. 53:680, 1955.

56. Green, H.; Sawyer, J. L., and Leopold, I. H.: Investigation of Corticosteroids in the Aqueous Humor of Normal Animal Eyes: II. Further Observations, Am. J. Ophth. 39:871, 1955.

57. Green, H.; Kromau, H. S., and Leopold, I. H.: Corticosteroids in the Aqueous Humor of the Rabbit; Paper Chromatography, A. M. A. Arch. Ophth. 54:853, 1955.

58. Foss, R. H.: Local Application of Diamox: An Experimental Study of Its Effect on the Intraocular Pressure, Am. J. Ophth. 39:336, 1955.

59. Gloster, J., and Perkins, E. S.: Effect of Carbonic Anhydrase Inhibitor (Diamox) on Intraocular Pressure of Rabbits and Cats, Brit. J. Ophth. 39:647, 1955.

60. Green, H., and Leopold, I. H.: Effects of Locally Administered Diamox, Am. J. Ophth. 40:137 (Nov., Pt. II) 1955.

61. Kinsey, V. E.; Palm, E., and Cavanaugh, G. A.: Mechanism of Posterior and Anterior Aqueous Humor Formation, Acta XVII Conc. Ophth. (1954) p. 1572, 1955.

62. Friedenwald, J. S.: Ascorbic Acid and Aqueous Flow, Acta XVII Conc. Ophth. (1954) p. 1598, 1955.

63. Böhringer, H. R., and Schelling, F.: The Ascorbic Acid Content of the Normal Aqueous and Aqueous with Inflammation in Man, Ophthalmologica 129:275, 1955.

64. Bárány, E. H., and Langham, M. E.: On the Origin of the Ascorbic Acid in the Aqueous Humor of Guinea Pigs and Rabbits, Acta physiol. scandinav. 34:99, 1955.

65. Langham, M. E.: The Use of Ascorbic Acid to Measure the Rate of Flow of Plasma Through the Ciliary Processes, J. Physiol. 130:1, 1955.

66. Ridge, J. W.: Sodium and Chloride of the Aqueous Humour of Normal and Scorbutic Guinea Pigs, Brit. J. Ophth. 39:534, 1955.

67. Auricchio, G.: Present Views on the Physiology of Intra-Ocular Fluids: I. Current Status of the Theory of Secretion, Boll. ocul. 34:257, 1955.

68. Auricchio, G.: Present Views on the Physiology of Intra-Ocular Fliuds: II. Determination of the Rate of Aqueous Humor Outflow, Boll. ocul. 34:277, 1955.

69. Auricchio, G.: Present Views on the Physiology of Intra-Ocular Fliuds: III. Origin of Ascorbic Acid in Intra-Ocular Fliuds, Boll. ocul. 34:287, 1955.

70. Bárány, E. H.: Studies on Aqueous Outflow, Acta XVII Conc. Ophth. (1954) 3:1546, 1955.

71. Bárány, E. H., and Woodin, A. M.: Hyaluronic Acid and Hyaluronidase in the Aqueous Humor and the Angle of the Anterior Chamber, Acta physiol. scandinav. 33: 257, 1955.

72. Tagaki, Y., and Kujo, G.: Studies on the Activity of Hyaluronidase in the Uvea and the Aqueous Humor, Acta Soc. ophth. japon. 59:167, 1955.

73. Sutcliff, F. P., and Hamilton, R. H.: Variations in Concentration of Ions in Aqueous Humor, Following Paracentesis in the Contralateral Eye, Am. J. Ophth. 39: 517, 1955.

74. Auricchio, G.: Contribution to the Knowledge of the Physiology of Intraocular Fluids: The Behavior of the Alkaline Reserve in Some Experimental Conditions, Gior. ital. oftal. 8:103, 1955.

75. Seki, S.: Studies on the Content of Urea in the Aqueous Humour: II-VI, Acta Soc. ophth. japon. 59:275, 1955.

76. Ito, Y.: Biochemical Studies on Accommodation: II. Pyruvate in the Aqueous Humour, Acta Soc. ophth. japon. 59:272, 1955.

77. Inamochi, J.: Fluorescein Permeability of the Blood Aqueous Barrier, Acta Soc. ophth. japon. 59:98, 1955.

78. Viikari, K.: Studies of the Cholinesterase Activity of the Aqueous Humor in Man and Some Animals, with Special Reference to the Influence of Certain Anticholesterases on It, Ann. med. exper. et biol. Fenniae (Supp. 4) 33:1, 1955.

79. Ross, E. J.: Insulin-like Effect of Anterior Pituitary Extract in Accelerating the Transfer of Glucose Across the Blood-Aqueous Barrier, J. Physiol. 127:247, 1955.

80. Kishimoto, M.; Ito, M.; Nakajima, N., and Hyodo, K.: Effect of Antuiturin upon the Thiamine Content of the Spinal and Intraocular Fluids, Acta Soc. ophth. japon. 59: 34, 1955.

81. Schmerl, E.; Dietz, A. A., and Steinberg, B.: Mechanism of Miopiesin Formation, Am. J. Ophth. 39:684, 1955.

82. Schmerl, E., and Steinberg, B.: Quantitative Effects of Miopiesin and Hyperpiesin on Intraocular Pressure, Am. J. Ophth. 39:547, 1955.

83. D'Ermo, E.: Blood Aqueous Barrier Permeability During Anaphylactic Shock in the Rabbit, Boll. ocul. 34:138, 1955.

84. Chodos, J. B.: Effect of Phenylbutazone and Synopen on the Blood-Aqueous Barrier in Rabbits, Internat. Arch. Allergy 7:92, 1955.

85. Küchle, H. J.: Experimental Basis for an Optimal PAS Therapy in Ophthalmology: II. The PAS Content of Aqueous Humor in Different Types of Local Administration and Under the Effect of Measures for Increasing the Permeability of the Cornea and of the Blood Aqueous Barrier, von Graefes Arch. Ophth. 156:261, 1955.

86. Küchle, H. J.: Experimental Basis for an Optimal PAS Therapy in Ophthalmology: III. PAS Content in Aqueous Humor of Eyes Changed by Inflammation and in Different Organic Tissues, von Graefes Arch. Ophth. 156:272, 1955.

87. Steiger, R. M.; Böhringer, H. R., and Wunderly, C.: Quantitative and Electrophoretic Studies of Newly Formed Aqueous Humor in Human Eyes, Klin. Monatsbl. Augenh. 126:490, 1955.

88. Milkman, R. D., and Kennedy, D.: Modification of Spectral Sensitivity by the Lens, Am. J. Physiol. 183:645, 1955.

89. François, J.; Rabaey, M., and Wieme, R. J.: New Method for the Fractionation of Lens Proteins, A. M. A. Arch. Ophth. 53:481, 1955.

90. Francois, J.; Rabaey, M.; Wieme, R., and Evens, L.; Electrophoretic Study of the Effect of pH on Lens Proteins, Bull. Soc. belge opht. 108:658, 1955.

91. Szent-György, A.: Fluorescent Globulin of the Lens, Biochim. et biophys. acta 16:167, 1955.

92. Stemmarmann, W., and Wallner, A.: Electrophoretic Studies on the Development of the Lens of the Chick, von Graefes Arch. Ophth. 156:630, 1955.

93. Fromageot, P., and Sivehis, J.: Isolation of a Homogenous Fraction of the Albumoid of Bovine Lens, Compt. rend. Acad. sc. 240:1730, 1955.

94. Sivehis, J.; Fromageot, P., and Petit, L.: Protein Composition of Albuminoid of the Crystalline Lens of Cattle, Compt. rend. Acad. sc. 241:1345, 1955.

95. Fromageot, P., and Prioux, H.: Demonstration of Proteases in the Lens of the Rat, Compt. rend. Acad. sc. 241:513, 1955.

96. Wieme, R. J., and Kaminski, M.: Study of the Antigenic Composition of Aqueous Extracts of Bovine Lens, Bull. Soc. chim. biol. 37:247, 1955.

97. Ujejski, L., and Glegg, R. E.: Carbohydrates in Thryglobulin and the Lens Capsule, Canad. J. Biochem. & Physiol. 33:199, 1955.

98. Kinoshita, J. H.: Carbohydrate Metabolism of Lens, A. M. A. Arch. Ophth. 54: 360, 1955.

99. Mandel, P., and Klethi, J.: Presence of a System of Direct Oxidation of Glucose-6-Phosphate in the Lens, Compt. rend. Acad. sc. 241:710, 1955.

100. Green, H.; Bocher, C. A., and Leopold, I. H.: Anaerobic Carbohydrate Metabolism of the Crystalline Lens: I. Glucose and Glucose-6-Phosphate, Am. J. Ophth. 39: 106 (Feb., Pt. II) 1955.

101. Green, H.; Bocher, C. A., and Leopold, I. H.: Anaerobic Carbohydrate Metabolism of the Crystalline Lens: II. Fructose Diphosphate, Am. J. Ophth. 39:113 (Feb., Pt. II) 1955.

102. Green, H.; Bocher, C. A., and Leopold I. H.: Anaerobic Carbohydrate Metabolism of the Crystalline Lens: III. Triosephosphate, Phosphoglycerate, and Phosphoenolpyruvate, Am. J. Ophth. 40:237 (Nov., Pt. II) 1955.

103. Dische, Z., and Ehrlich, G.: The Breakdown of Glucose and Its Phosphoric Esters in the Bovine Lens Capsule, Am. J. Ophth. 39:99 (Feb., Pt. II) 1955.

104. Dische, Z.: Metabolic Processes in the Lens Capsule of Beef and Rabbit, Acta XVII Cone. Ophth. 2:977, 1955.

105. Hauschildt, J. D.; Harris, J. E., and Nordquist, L. T.: Changes in the Phosphate Fractions of the Lens Under Various Conditions Which Influence Cation Transport, Am. J. Ophth. 39:155 (Feb., Pt. II) 1955.

106. Ozaki, S.: Energy Development in the Lens and Its Role in the Regulation of Osmotic Pressure, Acta Soc. ophth. japon. 59:621, 1955.

107. Schwartz, B., and Leinfelder, P. J.: Ascorbic-Dehydroascorbic Acid as an Oxidation-Reduction System: In the Maintenance of the Metabolism of Lens Cultured in Vitro; I. Weight and Cation Equilibrium, Am. J. Ophth. 40:224 (Nov., Pt. II) 1955.

108. Harris, J. E.; Hauschildt, J. D., and Nordquist, L. T.: Transport of Glucose Across the Lens Surfaces, Am. J. Ophth. 39:161 (Feb., Pt. II) 1955.

109. Kleifeld, O.; Müller, H. K.; Hoekwin, O.; Areus, P.; Fuchs, R., and Dardenne, U.: The Importance of Capsule and Epithelium in Phosphate Uptake by the Lens, von Graefes Arch. Ophth. 156:443, 1955.

110. Kliefeld, O.; Müller, H. K.; Dardenne, U.; Fuchs, R.; Hoekwin, O., and Areus, P.: The Breakdown of P^{32} into Electrophoretically Separable Phosphate Fractions by the Normal Lens, von Graefes Arch. Ophth. 156:453, 1955.

111. Kleifeld, O.; Hoekwin, O., and Areus, P.: On the Question of an Action Metabolism of the Lens von Graefes Arch. Ophth. 156:467, 1955.

112. Hans, W.; Hoekwin, O., and Kleifeld, O.: Determination of the O_2 Consumption of the Lens by Polarography, von Graefes Arch. Ophth. 157:72, 1955.

113. Schwarz, Y., and Goldberg, L.: Galactose-1-Phosphate in Galactose Cataract, Biochem. et biophys. acta 18:310, 1955.

114. Tojo, H.: Developmental Mechanism in Galactose Cataract, Acta Soc. ophth. japon. 59:933, 1955.

115. Van Heyningen, R.; Pirie, A., and Blackwell, J.: Effect of Cocarboxylase on the Development of Galactose Cataract in Rats, Brit. J. Ophth. 39:37, 1955.

116. Nordmann, J., and Mandel, P.: Enzyme Studies in Radiation Cataract, Am. J. Ophth. 40:871, 1955.

117. Pirie, A.; Van Heyningen, R., and Flanders, P. H.: Changes in Lens During the Formation of X-Ray Cataract in Rabbits: III. Phosphates, Biochem. J. 61:341, 1955.

118. Daisley, K. W.: Synthesis of Glutathione by Normal and X-Irradiated Lens, Biochem. J. 60:XL, 1955.

119. Telkkä, A.: Histochemically Demonstrable Sulfhydryl Groups in the Ocular Tissues of Normal Rat and After X-Ray Irradiation with Special Reference to the Lens, Acta path. et microbiol. scandinav. 36:21, 1955.

120. Müller, H. K.; Kleifeld, O.; Fuchs, R.; Dardenne, H.; Hoekwin, O., and Areus, P.: The Breakdown of Radiophosphorus into Electrophoretically Separable Phosphate Fractions of the Lens, Relative to Age and to Poisoning with Naphthalene, Dinitrophenol and Alloxan, von Graefes Arch. Ophth. 156:460, 1955.

121. Odabara, H.: Mechanism of Cataract Development in Alloxan Diabetic Animals, Acta Soc. ophth. japon. 59:576, 1955.

122. Orsalesi, F.; Pirodda, A., and Miglior, M.: Possibility of Interaction Between the Lens Protein and Other Proteins, Gior. ital. oftal. 8:272, 1955.

123. Orsalesi, F.; Miglior, M., and Pirodda, A.: Lens Opacities Caused in Vitro by Basic Proteins, Gior. ital. oftal. 8:281, 1955.

124. Orsalesi, F.; Pirodda, A., and Miglior, M.: Permeability of Isolated Capsule of Calf and Horse to Electronegative and Electropositive Proteins, Gior. ital. oftal. 8:265, 1955.

125. Orsalesi, F., and Miglior, M.: Influence of Blood Serum on the Permeability of the Lens Capsule to Glucose, Gior. ital. oftal. 8:202, 1955.

126. Orsalesi, F., and Pirodda, A.: Investigation on the Permeability of the Lens Capsule to the Protein of Blood Serum, Gior. ital. oftal. 8:191, 1955.

127. Tron, L. S., and Tartakovskaja, R. Z.: The Action of Certain Hormones on the Lens, Vestnik oftal. 34:20, 1955.

128. Cascio, G., and Ponte, F.: Investigations on the Permeability of the Lens Capsule in Some Experimental Retinopathies, Rass. ital. ottal. 24:33, 1955.

129. Kinsey, V. E.; Wachtl, C.; Constant, M. A., and Camacho, E.: Studies on the Crystalline Lens: VI. Mitotic Activity in the Epithelia of Lenses Cultured in Various Media, Am. J. Ophth. 40:216 (Nov., Pt II) 1955.

130. Davson, H.: Nutrition of the Lens by Way of the Aqueous Humour, Acta XVII Conc. Ophth. (1954) 3:1977, 1955.

131. Woodin, A. M., and Boruchoff, S. A.: Particle Interaction in Solutions Derived from Ox Vitreous Humor, J. Biophys. & Biochem. Cytol. 1:489, 1955.

132. Jensen, C. E.; Koefoed, J.; Vilstrup, G., and Vilstrup, T.: Hyaluronic Acid: X. Flow of Elasticity and Flow Potentials in the Corpus Vitreum, Acta ophth. 33:395, 1955.

133. Dische, Z., and Zelmanes, G.: Polysaccharides of the Vitreous Fibers, A. M. A. Arch. Ophth. 54:528, 1955.

134. Laurent, T. C.: Studies on Hyaluronic Acid in the Vitreous Body, J. Biol. Chem. 216:263, 1955.

135. Varga, L.: Studies on Hyaluronic Acid Prepared from the Vitreous Body, J. Biol. Chem. 217:651, 1955.

136. Brunish, R.; Rowen, J. W., and Irvine, S. R.: Proteins and Hyaluronic Acid in Beef Vitreous Humor, Tr. Am. Ophth. Soc. 52:369, 1955.

137. Irvine, R. S.; Brunish, R., and Rowen, G. W.: Studies on the Vitreous Body, Am. J. Ophth. 39:100, 1955.

138. Südhof, H., and Schweer, G.: Studies on Glucosamine of the Vitreous Body: I. Glucosamine Determination in the Bovine Vitreous Body, von Graefes Arch. Ophth. 156:427, 1955.

139. Südhof, H., and Schweer, G.: Studies on the Glucosamine of the Vitreous Body: II. The Content in Glucosamine of the Vitreous in Relation to Age, von Graefes Arch. Ophth. 157:85, 1955.

140. Cristiansson, J.: The Influence of Hyaluronidase on the Rate of Diffusion in the Vitreous Body in Vivo: Experiments with Radioactive Phosphate on Rabbit Eyes, Acta ophth. 33:571, 1955.

141. Schimek, R. A., and Steffensen, E. H.: Vitreous Hemorrhage Absorption: Experimental Study on Rabbit Eyes of the Effects of Intravitreal Hyaluronidase and Streptokinase-Streptodornase and on the Influence of ACTH and Cortisone, Am. J. Ophth. 39:677, 1955.

142. Dardenne, U., and Breuer, H.: The Occurrence of Steroid Hormones in the Vitreous, von Graefes Arch. Ophth. 156:283, 1955.

143. Donn. A.: Ultrasonic Wave Liquefaction of Vitreous Humor in Living Rabbits, A. M. A. Arch. Ophth. 53:215, 1955.

144. Vilstrup, G., and Kornerup, V.: Protein Fractions in Corpus Vitreum Examined by Paper Electrophoresis, Acta ophth. 33:17, 1955.

145. Rushton, W. A. H.; Campbell, F. W.; Hagins, W. A., and Brindley, G. S.: The Bleaching and Regeneration of Rhodopsin in the Living Eye of the Albino Rabbit and of Man, Optica acta 1:183, 1955.

146. Campbell, F. W., and Rushton, W. A. H.: Measurement of the Scotopic Pigment in the Living Human Eye, J. Physiol. 130:131, 1955.

147. Weale, R. A.: Bleaching Experiments on Eyes of Living Guinea Pigs, J. Physiol. 127:572, 1955.

148. Pitt, G. A. J.; Collins, F. D.; Morton, R. A., and Stok, P.: Studies on Rhodopsin: 8. Relinylidenemethylamine, an Indicator Yellow Analogue, Biochem. J. 59:122, 1955.

149. Morton, R. A., and Pitt, G. A. J.: Studies on Rhodopsin: 9. pH and the Hydrolysis of Indicator Yellow, Biochem. J. 59:128, 1955.

150. Wald, G.; Brown, P. K.; Hubbard, R., and Oroshnik, W.: Hindered Cis Isomers of Vitamin A and Retinene: The Structure of the Neo-B Isomer, Proc. Nat. Acad. Sc. 41:438, 1955.

151. Hubbard, R.: Retinene Isomerase, Fed. Proc. 14:229, 1955.

152. Wald, G.; Brown, P. K., and Smith, P. H.: Iodopsin, J. Gen. Physiol. 38:623, 1955.

153. Weale, R. A.: Bleaching Experiments on Eyes of Living Grey Squirrels (Sciurus Carolinensis Leucotis), J. Physiol. 127:587, 1955.

154. Arden, G. B., and Tansley, K.: The Spectral Sensitivity of the Pure-Cone Retina of the Grey Squirrel (Sciurus Carolinensis Leucotis), J. Physiol. 127:592, 1955.

155. Arden, G. B., and Tansley, K.: The Spectral Sensitivity of the Pure-Cone Retina of the Souslik (Citellus Citellus), J. Physiol. 130:225, 1955.

156. Wald, G., and Burg, S. P.: Crustacean Vitamin A, Fed. Proc. 14:300, 1955.

157. Lambertsen, G.: A Possible New Vitamin A_3 Isomer in the Eye of Crustaceans, Nature, London 176:553, 1955.

158. Krinsky, N.: Esterification of Vitamin A in Vitro, Fed. Proc. 14:88, 1955.

159. Noell, W. K.: Metabolic Injuries of the Visual Cell, Am. J. Ophth. 40:60 (Nov., Pt. II) 1955.

160. Francis, C. M.: Lipids in the Retina, J. Comp. Neurol. 103:355, 1955.

161. Gourewitch, A.: Investigation on the Formation of Orthophosphate in the Illuminated Retina, Compt. rend. Soc. biol. 149:918, 1955.

162. Hebb, C. O.: Choline Acetylase in Mammalian and Avian Sensory Systems, Quart. J. Exper. Physiol. 40:176, 1955.

163. Coulombre, A. J.: Correlations of Structural and Biochemical Changes in the Developing Retina of the Chick, Am. J. Anat. 96:153, 1955.

164. Rosen, D. A.; Maengwyn-Davies, G. D.; Becker, B.; Stone, H. H., and Friedenwald, J. S.: Xanthuronic Acid Excretion Studies in Diabetics With and Without Retinopathy, Proc. Soc. Exper. Biol. & Med. 88:321, 1955.

165. Maengwyn-Davis, G. D.; Becker, B.; Rosen, D. A., and Friedenwald, J. S.: A Comparison of the Acetylating Capacity of Normals and Diabetics With and Without Retinopathy, Bull. Johns Hopkins Hosp. 96:150, 1955.

166. Hayano, S.: Relation Between the pH of the Retina and Visual Cycle, Acta Soc. ophth. japon. 59:599, 1955.

167. Koride, Y.: Experimental Studies of the Regeneration of Rhodopsin: I-III, Acta Soc. ophth. japon. 59:605, 1955.

168. Kanapa, E. M.: Euphansiopsin, a New Photosensitive Pigment from the Eyes of Euphansiid Crustaceans, Nature, London 175:996, 1955.

169. Matsuralia, T.: Interrelationship of Histochemical and Electroretinographic Changes in the Retina, Acta Soc. ophth. japon. 58:836, 1955.

170. Smelser, G. K., and Ozanics, V.: Further Studies on the Nature of the Exophthalmos-Producing Principles in Pituitary Extracts, Am. J. Ophth. 39:146 (Feb., Pt. II) 1955.

171. Weitzel, G.; Buddecke, E.; Fretzdorff, A. M.; Strecker, F. J.; Roester, U.: Structure of Zinc Compound in Tapetum Lucidum of Dog and Fox, Ztschr. physiol. Chem. 299:193, 1955.

172. Baron, J. B.; Bessineton, J. C.; Guillon, S.; Cabau, N.; Nham, L., and Monestier, F.: Variations of the Levels of Organic and Mineral Ions in the Eye, Brain, and Serum in Light and in Darkness, Ann. biol. clin. 13:98, 1955.

173. Terner, I. S.; Leopold, I. H., and Eisenberg, I. J.: The Radioactive Phosphorus (P^{32}) Uptake Test in Ophthalmology: A Review of the Literature and Analysis of Results in 262 Cases of Ocular and Adnexal Pathology, A. M. A. Arch. Ophth. 55:52, 1956.

News and Comment

SOCIETY NEWS

Société francaise d'ophtalmologie.—The French Society of Ophthalmology held its 63d yearly meeting in Paris, May 6 through 10, 1956. There were as usual a great number of foreign members, mostly from Europe but also from the Western Hemisphere.

This year the main paper (*rapport*) was by Paul Bregeat, on Papilledema.

This is a very large book, of 862 pages with 170 figures and 14 colored plates. (This book is distributed free of charge to all members of the French Society. Nonmembers can obtain it by applying either to the Treasurer of the Society: Dr. J. Charpentier, 28, rue Vignon, Paris 9, or to the publisher: Masson & Cie, 120 boulevard Saint-Germain, Paris 6. The price of the volume is 8000 francs.) It is divided in four parts. The first contains chapters on anatomy and physiology of the disc, the optic nerve, and cerebral vascularisation; on general physiopathology of edema throughout the body, and on relationship between cerebral edema and papilledema. The second part is devoted to the semiology of papilledema and to the clinical study of both real papilledema and false papilledema.

The third part is a careful study of what the author calls *œdèmes papillaires accompagnés*; the fourth, to *œdèmes papillaires purs*. Brejeat has classified in the last group choking of the disc due to increased intracranial pressure, and in the former he includes all cases when the optic fibers are not only bathed in edema but have also suffered and been damaged.

On Monday, May 7, the papers concerned the orbit, the lids, and the adnexae.

Larmande (Algiers) reported on tumors of the orbital roof and stressed particularly their x-ray picture. Dollfus, Legrand, and Baclesse (Paris) observed, in a child 4 years old, an eosinophilic granuloma of the frontal bone with orbital symptoms, which had destroyed the bone but responded promptly to roentgen therapy. One month after this treatment was started the x-rays showed that the bone was beginning to be restored. Paufique, Etienne, and Charleux (Lyon) observed a patient with Recklinghausen's disease. The roof of the orbit had completely disappeared, and there was a pulsating exophthalmos due to the transmission of the cerebral pulse. This disappeared after surgical restoration of the bone of the roof. Desvignes and Dagorne (Paris) reported on two patients with orbital cylindroma. They both died, the roentgentherapy and surgery proving useless. Litricin and Jovicevic (Belgrade) stressed the diagnostic difficulties of pseudotumors of the orbit. Hervouet (Nantes) and Tessier (Paris) found a new technique satisfactory when operating on ptosis. Taking into account the normal antagonism of the levator and orbicularis, they resect the levator but also remove a considerable amount of the orbicularis in the upper lid. Valière-Vialeix and Robin (Limoges) observed a patient for many years who had initially a pemphigus of the conjunctiva. Eventually he developed an epithelioma under the upper lid. Ayberk (Istanbul) observed a man, 70 years old, who developed, suddenly and simultaneously, sarcomas in all four lids. He was treated with mustard gas and nitrogen and the tumors disappeared. There has been no recurrence in the last four years. Calmettes, Deodati, Planel, and Bec (Toulouse) experimented on 50 guinea pigs; after castration they were given sexual hormones; the lacrymal glands underwent changes: hypotrophy in the male, hypertrophy in the female. Compensating hormonal treatment corrected these anomalies. This may be of interest in the pathogeny and treatment of Sjögren's disease. Algan and Cordier (Nancy) reported on two cases of dacryoadenitis in sarcoïdosis.

On Wednesday, May 9, the papers were on the optic nerve and on amblyopia. François, Woilez, and Asseman (Lille) reported four cases of optic neuritis which, during recovery, had a ring scotoma. They discussed the pathogeny of

this unusual visual field. Torres-Estrada (Mexico) discussed the pathogeny of optic nerve cupping in glaucoma. Streiff and Deller (Lausanne) observed a case of retrobulbar neuritis with complete loss of central vision in a patient with Q fever. Blagojevic and Arambasic (Belgrade) examined 250 patients with tuberculous meningitis; all were examined ophthalmoscopically every month, and in 40 cases there was a postmortem examination; the authors discuss the pathogenesis of optic nerve involvement in this disease. Viallefont, Boudet, and Jaulmes (Montpellier) stressed that in leukemia, besides implication of the retina, the optic nerves were also often involved; out of 90 patients, 44% showed anomalies of the nerve head. Thomas (Nancy) discussed the cause of amblyopia. This can be located anywhere from the retina to the visual cortex; treatment must be different according to the site of trouble. Sevrin (Charleroi) and Cuppers (Giessen) also discussed amblyopia but more especially when nystagmus is also present. They reported on some very good results when surgery was completed by the Cuppers technique of amblyopia treatment. Grancini and Cenacchi (Varèse) observed anomalies of the EEG in children with amblyopia. Sedan (Marseilles) found it possible to help patients of very poor vision by attaching fluorescent papers to different objects such as toilet articles, knife, fork, and working utensils. De Jaeger and Bernolet (Bruges) reported on the treatment of amblyopia ex anopsia by a new technique; they use the normal physiologic connexion of motor and sensory function.

Sunday, May 6, was devoted to free papers. Zuccolo (Lugano) discussed retinal cysts. I. Stankovic (Belgrade) examined 116 patients with pellagra but did not observe the ocular lesions usually found in that disease. Durix and Gallet (Casablanca) reported on ocular lesions observed after the use of tear gas by the police; these lesions are particularly to be feared when trachoma is also present. Fritz (Brussels) observed that before acetazoleamide (Diamox) brings down the ocular tension there is a phase of marked venous congestion. Ourgaud (Marseilles) measured the visual acuity by a new method, in flickering light. Von Berger, Fadiga, and Pupilli (Bologna) studied the electric reponse of the cerebellum in the cat during visual stimulation. Kapuscinski, Ogielski, and Ogielska (Wroclaw) experimented on allergy in iridocyclitis; they consider that the bacterial and toxic factor is the most important.

Thursday, May 10, was also given over to "free papers." Favory and Lagraulet (Paris) stressed the frequency of retinal and optic nerve involvement in onchocercosis. Bischler and Franceschetti (Geneva) discussed different types of pigmentation of the disc. Appelmans and Michiels (Louvain) reported on newborn children with lesions of the optic nerve or retina (atrophy and gliomatosis); there were no traumas, and they consider the cause to be toxic. Zanen, Meunier, and P. Coppez (Brussels) studied the chromatic and achromatic threshold of central vision in certain diseases of the optic nerve; incidentally, they questioned the usual opinion that green and red are first impaired. Dubois-Poulsen, Guillaume, and Magis (Paris) observed a patient with Parkinson's disease whose anterior choroidal artery had been ligated. There was a lateral hemianopsia which in no way differed from one observed after a lesion posterior to the lateral geniculate body. This is contrary to expectation and suggests many questions. Charamis (Athens) stated that when the retina is detached, after an intraocular foreign body, the result is poor when the foreign body is extracted in the usual way and the retinal tear is closed; on the contrary, he obtained a complete cure in seven cases when he removed the foreign body through the retinal tear. Georgiades (Salonica) discussed the different types of cataract associated with heterochromia (69 cases of Fuchs disease, 9 cases of hypochromia, and 6 cases of hyperchromia); in all cases there was a marked sclerosis of the blood vessels in the iris. Bouzas (Athens) studied the aqueous in ocular diseases due to leprosy. There often were cytologic changes and increased protein, but he never found Hansen's bacillus. Necdet Sezer (Istanbul) has tried to culture the trachoma virus; he obtained small round white dots of a structure very near that of trachoma follicles but without any inclusions.

During the five-day meeting there were operating sessions and clinical demonstrations in many hospitals. On Tuesday, May 8, the banquet was held in a restaurant half way up the Eiffel Tower, with Dr. Hambresin, of Brussels, presiding. The next afternoon an excursion was organized to three castles in the vicinity of Paris: Marais, Saint-Jean-de-Beauregard, and Courson.

Several other ophthalmological organizations met during the same week: the International Organization Against Trachoma, the Society for Prevention of Blindness, and the Entretiens Therapeutiques. The International Council of Ophthalmology met just before the French Society, on May 5.

The 64th meeting of the French Society of Ophthalmology will be held in Paris May 12 through 16, 1957.

Books

Blur of the Retinal Image. By Glenn A. Fry, M.D. Price, 3.50. Pp. 120. The Ohio State University Press, Columbus, Ohio, 1955.

This small book is a fairly technical discussion of how the distribution of the intensity of light within the retinal image can be determined. The first-order effects and roles played by out-of-focus images, diffraction, axial chromatic aberration, chromatic dispersion, small astigmatism, spherical aberration, and pupil size in this distribution are developed analytically. The consideration deals with point sources of light, the straight line boundary between light and dark areas, and luminous line objects. It is assumed at the outset that the simple distribution of light within the image can be described by the normal error-distribution curve. From this as a basis an "index of blur" is defined. Much of the book then is concerned with determining the variation of this index under the influence of the different factors listed above. The discussion of the characteristics of the retinal image due to diffraction alone in out-of-focus images is particularly interesting. Similarly, the chapter on the relative roles played by diffraction and spherical aberration on the out-of-focus images is enlightening. According to the calculations, the eye with spherical aberration is nearly equivalent to an eye free from spherical aberration, if one considers the point of best focus to lie about 0.26 mm. in front of the paraxial focus, which is equivalent to 0.72 D. The caustic surface appears unrelated to the concentration of light in the image.

The author summarizes by stating as follows: "It is believed that the calculations presented herein have sufficiently demonstrated that simple blur circle theory gives an approximation to the truth, even in the case of spherical aberration, which completely justifies the classical use to which it has been put in dealing with accommodation and refraction, and justifies the effort which is generally made by refractionists to work within a precision of an eighth to a quarter of a diopter." The analysis presented is deemed needed in investigations of perception of fine detail. The reviewer is certain also that the author would be the first to admit that generally the analysis is only a first approximation to the case of the average eye, where the lack of axial symmetry of the refractive surfaces and the ever-present astigmatic irregularities of those surfaces tend to complicate the distribution of light in out-of-focus images. One only needs to accommodate voluntarily while observing a luminous object against a black background (say, the moon at night) to see several separate higher concentrations of light. Apart from this criticism, Dr. Fry's book is a valuable contribution and it will provide a reference text for those concerned with the finer aspects of the problem of the retinal image.

The average reader without some background in optics and mathematics may find the book difficult to read. Probably there should have been more amplification of the discussion following the development of each topic.

The book is a photographic offset of typewritten material but is easily readable.

New Univis lenses with **25mm.** segments

from stock for your Rx!

TM. D-25

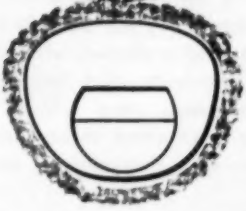
Note the identifying tinted line

Univis Nu-line D-25

The original Univis straight-top design—now in 25 mm. segment width when you need wider lateral field at near. *Also has special Nu-Line treatment developed for wide segment bifocals.* Prescribe for absolute security in the four vital areas of quality which *must* be built in by the manufacturer.

Univis CV6-25

You can now prescribe the original improved-type 3-foci multifocal with a 25 mm. segment—Univis. Highest standards of quality rigidly maintained. Rx for general purpose wear for 2.00 D reading add or greater.



Univis Nu-line CV 7-25

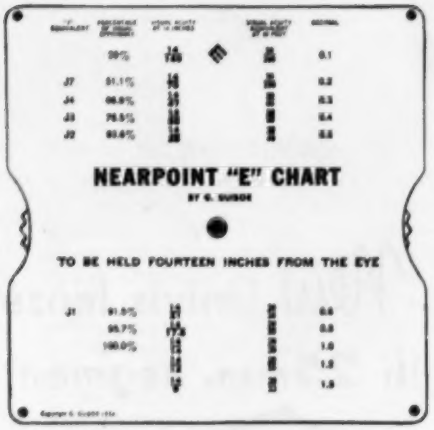
The popular Univis CV, specially treated to eliminate more than 80% of reflection and refraction by segment lines, now in 25 mm. segment width as well as in 23. Rx where reading add is greater than 2.00 D.

From stock, for wide lateral field at near and intermediate

UNIVIS

THE UNIVIS LENS COMPANY

Dayton, Ohio



New Near Chart

GUIBOR Symbol Near Chart now available in durable and soilproof plastic. Gives distance equivalents, as well as quantitative measurements for near vision. E Symbol understood by all.

Place your order now.

$3.25

DISPENSING SERVICE

Main Office:
111 N. Wabash Ave., at Wash.
Chicago, Illinois

Austin Belgard INC.

OPHTHALMIC OPTICIANS

WHOLESALE & SERVICE

Branch Office:
1139 Central Ave., Wilmette, Ill.

Rx

QUALITY SERVICE

Technical skills and services that will never let you down

DOW

Top-Grade Technical Performance

. . . a Safeguard for Your Professional Skills and Judgment.

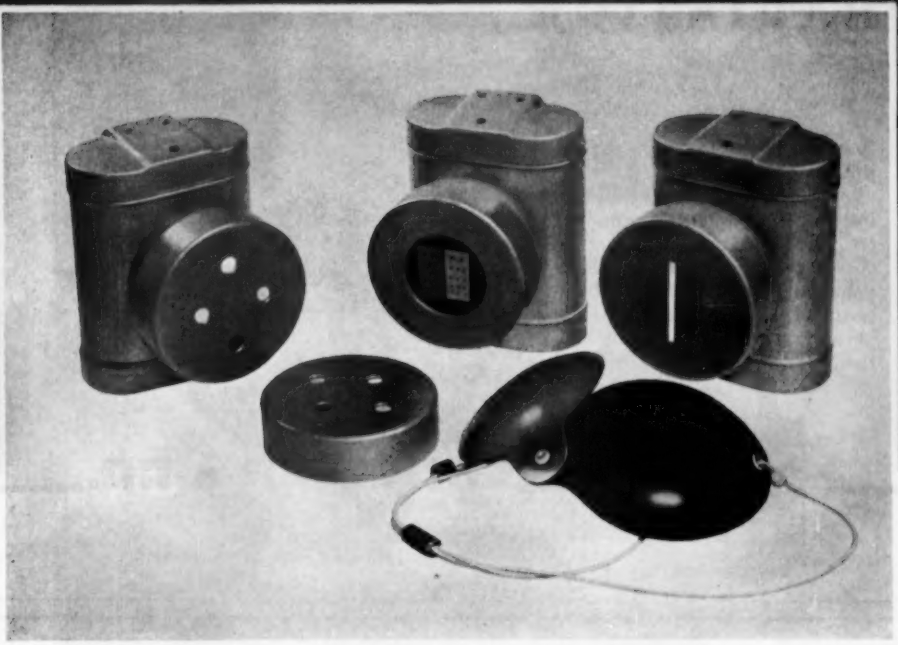
¶Your prescriptions, conceived from your rich fund of scientific knowledge laboriously developed through years of study, training and experience, are at the mercy of the dispensers who interpret them. They will be safe in the hands of Dow seasoned craftsmen, whose devotion to the exacting standards which your professional reputation demands, and which our firm has consistently maintained throughout its 31 years of existence, is a guarantee of complete patient satisfaction.

DOW OPTICAL CO.

W. E. DOW, Pres.

Chicago, Ill. Bloomington, Ill.

Clement Clarke Present



ILLUMINATED NEAR VISION TESTS

England's leading specialists in the manufacture of orthoptic equipment, including Moorfields Synoptophore, Lyle Major Amblyoscope, Ophthalmoscopes, Retinoscopes, Projection Perimeters, Slit Lamps, etc.

CLEMENT CLARKE

This convenient and inexpensive set is from the wide range of orthoptic equipment supplied by Clement Clarke of England. The set comprises one cycle torch glazed with an opal glass filter, hoods providing the Duochrome Near Vision Test, Worth's 4-Dot Test, and the Barlite Test, and a pair of complementary diplopia goggles. Particulars of other equipment are contained in the comprehensive Clement Clarke catalogue, sent on request.

INSTRUMENT DEPARTMENT: 63 WIGMORE STREET, LONDON, W.1, ENGLAND.

BETA RADIATION

FOR TREATMENT OF SUPERFICIAL CORNEAL DISEASES

You will be interested in the scientific data compiled in our booklet, "Radiation Therapy Sources." It includes clinical data, indications for treatment by irradiation, suggested dosage table, U. S. Atomic Energy Commission regulations, and a description of our improved M-1 Applicator. It's yours without obligation.

The M-1
Strontium-90 Applicator

Armamentarium

V MUELLER & CO.
330 South Honore Street
Chicago 12, Illinois
Rochester, Minn. • Dallas, Tex. • Houston, Tex.

A DRUG OF CHOICE IN GLAUCOMA THERAPY . . .

OPHTHALMOS

PILOCARPINE HCl.
Solutions
½%—1%—2%—4%

OFFER COMFORT AND CONTINUITY OF THERAPY IN THE TREATMENT OF THE *Glaucomas*

—UNDESIRABLE SIDE EFFECTS VIRTUALLY ELIMINATED—
RAPID PROLONGED ACTION

OPHTHALMOS Sterile Pilocarpine HCl. Solutions are prepared according to the Formulae developed by Robert R. Feinstein Research Associates

Prescribe in original 15cc. Dropper Sealed Bottles obtainable promptly through Prescription Pharmacies everywhere

OPHTHALMOS, INC. **UNION CITY, N. J.**

Distributed in Canada by Professional Sales Corp. • 5333 Queen Mary Road, Montreal 29, Quebec

Now you can do a visual field examination in 5 minutes!

(Developed by
David O. Harrington, M. D.
and Milton Flocks, M. D.)

Try the new Field Screener yourself at the 1956 meeting of the American Academy of Ophthalmology and Otolaryngology. Booths 103-105, Exhibition Hall, Palmer House, Chicago.

priced at only **$185**

Jenkel-Davidson
OPTICAL COMPANY

366 POST STREET • SAN FRANCISCO, CALIFORNIA

Specialists in ALL TYPES of Plastic and Glass Artificial Human Eyes Exclusively

MADE TO ORDER IN OUR OWN LABORATORY

Doctors are invited to visit

PREFERRED CASES CAREFULLY ATTENDED AND SATISFACTION GUARANTEED

EYES ALSO FITTED FROM STOCK

Plastic or Glass Selections Sent on Memorandum upon Request

Implants and Plastic Conformers in Stock

FRIED AND KOHLER, INC.

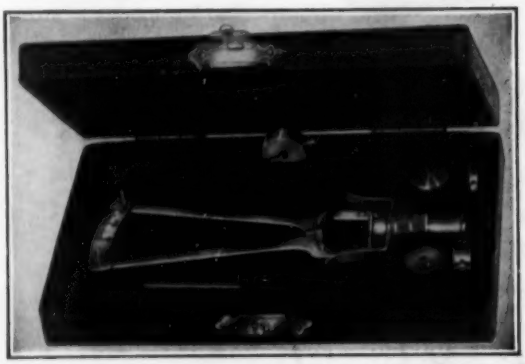
665 FIFTH AVENUE
near 53rd St.

NEW YORK, N. Y.
Tel. ELdorado 5-1970

E. B. Meyrowitz

SURGICAL INSTRUMENTS CO., INC.,
520 FIFTH AVENUE, NEW YORK 36, N. Y.

LONDON ***ESTABLISHED 1875*** **PARIS**



SCHIOTZ TONOMETER

IN STAINLESS STEEL

This instrument is made in strict accordance with the revised specifications adopted on March 8, 1952, by the Committee on Standardization of Tonometers of the American Academy of Ophthalmology and Otolaryngology, and is supplied in a dust-proof case which has separate recesses for the Instrument, Test Block, Weights, and Plunger.

As made in our own laboratories it preserves all of its original effectiveness for determining ocular tension with a new margin of accuracy, sturdiness, ease of handling and beauty of finish that is characteristic of instruments made by Meyrowitz.

It has been found satisfactory by many Ophthalmologists and meets all the specifications of the A.A.O.O. Tonometry Committee.

Approved by the Electrical Testing Laboratories, it leaves nothing to be desired from the standpoint of accuracy.

In case, with certification, each $55.00

See Us at the A.A.O.O. Convention—Palmer House, Chicago, Ill., Oct. 14-19, Booths 79 & 80

CASE HISTORY #3

Case History #3 explains the usefulness of Catarex Service in cases of bilateral cataract extractions. In this case, immediate vision was especially important, since the patient, a diabetic, lived alone and had to administer insulin to herself daily.

This case history emphasizes the fact that CATAREX T temporary cataract bifocals have proved themselves more versatile in aphakic cases *than any other form of temporary lens.* CATAREX T lenses are a simple answer to the old, complicated problem of easing the patient through the post-operative period.

Why not use this splendid service on *your* next cataract case? Your optician or laboratory will be happy to supply details.

CASE HISTORY – Mrs. Y. Y.

A 67-year-old diabetic female had a left extracapsular lens extraction on October 26, 1954, and one week later, November 2, 1954, had a right intracapsular lens extraction. The post-operative course was normal. Patient was discharged November 10.

11-18-54: The patient was given the right and left +10.00 CATAREX T (temporary) lenses which had been ordered for her soon after discharge from the hospital. That she could avail herself of such a service was important, for she lived alone and had to administer insulin to herself daily.

11-23-54: Manifest O.D. +12.00Dsph. = 20/70—. Manifest O.S. +12.00Dsph. = 20/70+. Rx: these in CATAREX T (temporary style) straight-top bifocals. Until these were received by the patient, she continued to wear the previous lenses. This policy was followed in all subsequent changes, so the patient was never without a pair of CATAREX T bifocals.

12-16-54: Manifest O.D. +12.00 = 20/20—; Manifest O.S. +13.00 = 20/30. Rx: these in CATAREX T bifocals.

1-6-55: Manifest O.D. +12.00Dsph. ○ +1.00Dcyl. ax 165 = 20/15+; Manifest O.S. +11.00Dsph. ○ +1.00Dcyl. ax 90 = 20/20—. Rx: these in CATAREX T bifocals.

1-14-55: Manifest O.D. +13.50Dsph. ○ +1.00Dcyl. ax 165 = 20/15; Manifest O.S. +12.50Dsph. ○ +1.00Dcyl. ax 90 = 20/20+. Rx: these in CATAREX T bifocals.

4-21-55: Manifest O.D. +13.00Dsph. ○ +1.00Dcyl. ax 180 = 20/15+; Manifest O.S. +13.00 Dsph. ○ +1.00 Dcyl. ax 150 = 20/20. Rx: these in CATAREX T bifocals.

7-18-55: Manifest O.D. +13.25Dsph. ○ +1.00Dcyl. ax 180= 20/15+; Manifest O.S. +13.00Dsph. ○ +0.75Dcyl. ax 135 = 20/20+; +2.75D add = Jg. 0.75 @ 13". Rx: these in permanent CATAREX D bifocals.

3-7-56: With permanent CATAREX D of 7-18-55, the right vision = 20/15+; the left vision = 20/20+.

The patient received her first pair of CATAREX T (temporary style) lenses eight days after discharge from hospital. She had six different pairs of temporary lenses during a period of eight months before the permanent CATAREX D bifocals were prescribed. Because she had useful vision throughout this period, there was no urgency to prescribe the permanent lenses. The importance of this is indicated by the fact that there was no need to change these lenses eight months after they were prescribed. The patient was not only made visually comfortable post-operatively and enabled to administer insulin to herself daily, but was spared the expenditure of the relatively large amounts which she could ill-afford, for several changes of the regular post-cataract bifocals, which would have been mandatory if the Catarex Temporary Service were not available to her.

CATAREX 'T'
TEMPORARY CATARACT BIFOCAL LENSES BY VISION-EASE

Manufacturers of a complete line of quality multifocals

His Best Friend Can't Tell

Your patients gain confidence in their public life knowing that others won't notice the artificial eye. They avoid feelings of inferiority in their personal life when best friends can't tell the difference.

Let our 105 years' experience help you perfectly match your patients' eyes. Our experienced men visit most areas regularly to fit your patients, custom-make eyes to match, and offer you technical assistance on difficult cases. Call or write our nearest office.

Complete Glass and Plastic Artificial Eye Service

- Made to order
- Selections from stock
- Eyes sent on memorandum same day order received
- Damaged or broken eyes accurately matched
- Fitted to all types of motility implants
- Implants, X-Ray therapy shields, foreign body locators
- Superior Quality—Finest Workmanship

Mager and Gougelman inc.

Serving the Profession Since 1851

30 N. Michigan Ave. Chicago 2, Illinois
510 Madison Ave. New York 22, N. Y.

CLEVELAND DETROIT KANSAS CITY MINNEAPOLIS ST. LOUIS BOSTON PHILADELPHIA PITTSBURGH WASHINGTON

Combine your own clinical experience with the knowledge of experts . . .

by reading

- **A.M.A. Archives of DERMATOLOGY**
- **A.M.A. Archives of PATHOLOGY**
- **A.M.A. Archives of INDUSTRIAL HEALTH**

Write To:
Subscription Department

American Medical Association
535 N. Dearborn Street
Chicago 10, Ill.

Of interest to you and your patients

THE MENACE OF ALLERGIES

WHAT WE KNOW ABOUT ALLERGY
by Louis Tuft, 12 pages, 15 cents

FOOD ALLERGY
by Samuel M. Feinberg, M.D., 6 pages, 10 cents

SKIN ALLERGY
by Samuel M. Feinberg, M.D., 6 pages, 10 cents

ASTHMA AND HAY FEVER
by Samuel M. Feinberg, M.D., 6 pages, 10 cents

AMERICAN MEDICAL ASSOCIATION
535 North Dearborn Street • Chicago 10 • Illinois

"LACRILENS"®

is a safe lens

The test of time proves that the "LACRILENS"® contact lens can be worn for long periods of time in aphakia and in all sports, including swimming. *Write For Brochure*

Obrig Laboratories Inc.
49 East 51st Street, New York 22, N. Y.

Manufacturers of all types of Contact Lenses

Branches in
PHILADELPHIA • MONTREAL • JOHANNESBURG

Most modern offices and factories are equipped with the best in scientific lighting facilities—but, they reflect on surfaces of glasses. These "GHOST" reflections are annoying and contribute to fatigue. MAY-O-LITE coated lenses can help eliminate these annoyances.

Complete Your Professional Service By Adding

The Best in Sight
MAY-O-LITE
Low Reflection Coating

MAY RESEARCH, INC., Box 1181, Minneapolis 1, Minn.

Night Driving Demands

Lestra-Lite

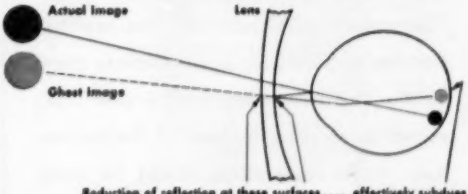
Reg. U.S. Pat. Off.

OPHTHALMIC LENS COATING

"There IS a Difference"

Reflections from eye-glass surfaces are most distracting while driving at night. Light reflected between the front and back lens surfaces and between the cornea and lens surfaces intensifies the glare of oncoming headlights, reducing the driver's visual discrimination. For easier, safer night driving, *always* prescribe Lestra-Lite.

Origin of Ghost Images in a Myopic Eye



Reduction of reflection at these surfaces.....effectively subdues ghost images on retina

FAST LESTRA-LITE SERVICE

Lenses processed and returned postpaid same day as received.

Write for half-coated Lestra-Lite Sample and Literature

PACIFIC UNIVERSAL PRODUCTS CORPORATION
158 Vista Avenue • Pasadena 8, California
130 Bush Street • San Francisco 4, California

THE BRAWNER G-L

A Modern Inclusion Implant

is the ideal implant for enucleation operations.

It is designed to simplify the operation—Eliminate muscle isolation—Minimize the possibility of extrusion or migration—Shorten post-operative hospital time—AND—

"It lets the patient decide" if he prefers a less-expensive conventional plastic reform eye with limited motility, or a Custom "Matching-back" prosthesis for adequate motility.

Full information, including description, prices and operative technique, as suggested by Dr. Brawner, will be sent on request.

Galeski Laboratory Inc.
737 East Main Street
Richmond 19, Virginia

NEW ORLEANS ACADEMY OF OPHTHALMOLOGY

The Seventh Annual meeting of the New Orleans Academy of Ophthalmology will be held in New Orleans in the Roosevelt Hotel—February 11-15, 1957, featuring "Symposium on Glaucoma." The registration fee of $75.00 includes associate membership in the Academy for the year 1957, as well as all other features of the convention. Hotel reservations should be made early by writing directly to the Roosevelt Hotel or to the Executive Secretary, P. O. Box 469, New Orleans, La.

℞ ORTHOPTICS

DVORINE ANIMATED FUSION CHARTS

A complete set of fusion charts in full color for office and home training, Base-In and Base-Out.

Exclusive Features:

- Animation
- Nine Groups of Charts
- Gay Colors
- Vertical Fusion Device
- Tracing Sheets
- Duo-Chrome Spectacles and Drawing Outfit

ALL IN ONE SET!
(with directions)

Price $12.00
Less 5%, if check accompanies order.

Available now on ℞ at your Ophthalmic Dispenser, American Optical Company and other Optical Supply Houses

SCIENTIFIC PUBLISHING CO.
Dept. A. 2328 Eutaw Place Baltimore 17, Md.

and other "dry eye" disturbances in the absence of infection...

in 15cc DROP-TAINER®

ISOPTO®–FRIN

Sterile Ophthalmic Solution

0.5% Methyl Cellulose U.S.P. and 0.12% Phenylephrine HCl U. S. P. Chlorobuntanol .15% added.

A non-greasy lubricant and decongestant for minor ocular irritation in the absence of infection.

advantages:

- *Sterile — Stable*
- *Preserved against contamination*
- *Non-greasy type emollient*
- *Readily miscible with tears*
- *Provides desirable cosmetic effect*
- *Comfortable and soothing to the eye*
- *Non-spillable, non-breakable*
- *Economically priced*

Usually provides prompt relief to those patients who complain vaguely of ocular disturbances such as "scratchy or itchy eyes," in the absence of any pathological conditions.

ISOPTO® designates Alcon Sterile Ophthalmic Preparations containing METHYL CELLULOSE.

Available from pharmacies and wholesale drug suppliers throughout the U.S.A. and Canada (Canadian distributors, Imperial Optical Co., Toronto)

Alcon laboratories, inc. • fort worth, texas

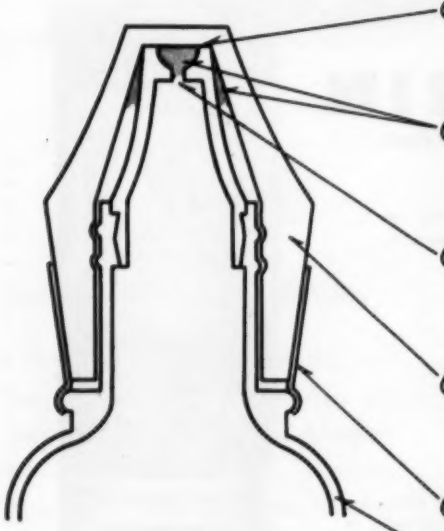
manufacturers of fine rhinologic and sterile ophthalmic specialties

AL-IF-1

New!

LACRIVIALS

ISO-SOL's brand of sterile polyethylene dropper bottles



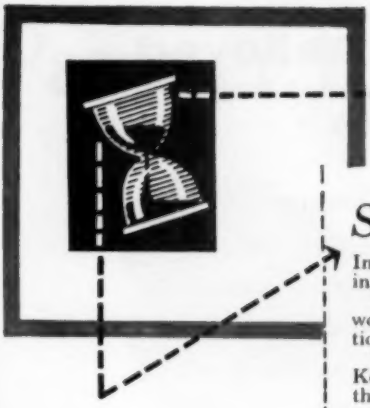
- **FLAT INNER SEALING SURFACES**
  - Leakproof
  - No crystallization
  - Avoids gumming
- **SELF-DISINFECTING DROPPER TIP**
  - Bacteriostatic containing medication, retained to disinfect tip between instillations
- **CONTROLLABLE DROPPING ORIFICE**
  - Cannot spill
  - Cannot leak
  - Sucks back unused medication
- **TRANSPARENT CAP**
  - Visible seal
  - Unbreakable
  - Color coded
- **TAMPER-PROOF OUTER SEAL**
- **LIGHTWEIGHT UNBREAKABLE BOTTLE**

Available:

Atropisol (atropine sulfate ½, 1, 2 & 4%), Bonazin (zinc sulfate ¼%), Bonmiotin (eserine salicylate ¼ & ½%, pilocarpine HCl 2%), Butacaine, Carbamiotin (carbachol ¾ & 1½%), Esromiotin (eserine salicylate ⅛, ¼ & ½%), Fluorescein, Phenylzin (zinc sulfate ¼%, phenylephrine HCl 0.08%), Pilomiotin (pilocarpine HCl ½, 1, 2 & 4%), Tearisol, Tetracaine.

Send for free sample box of Lacrivials

THE ISO-SOL CO., INC., ISO-SOL BROOKLYN 17, N. Y.



WESLEY CONTACT W/J LENSES JESSEN

Services to the Ophthalmologist

In the past four or five years tremendous strides have been made in the field of contact lenses.

The use of contact lenses for monocular aphakic cases alone would justify the study and fitting of contact lenses, not to mention the need of binocular aphakic cases.

To employ contact lenses as a diagnostic instrument is an aid. Keratoconus and irregular cornea cases can be often helped by the proper utilization of contact lenses.

Other uses of fitting contact lenses include: cases of distorted and discolored eyes (with cosmetic contact lenses); special activities, such as sports, in which normal spectacle lenses would be a handicap and dangerous; various subnormal vision cases . . . contact lenses can be used as the objective in the optical combination to create magnification.

The use of contact lenses for cosmetic purposes cannot be overlooked. There are many other applications for fitting contact lenses.

A knowledge of the various contact lenses should be a part of every ophthalmologist's practice.

Write us for descriptive literature and instructional material on the fitting of Wesley-Jessen contact lenses

THE PLASTIC CONTACT LENS COMPANY 59 E. MADISON ST. CHICAGO 2, ILLINOIS

Instruments of ophthalmology: The Costenbader Childrens Trial Frame

Parsons Development Program

Our San Francisco showrooms exhibit accepted apparatus for ophthalmology from throughout the world. In addition Parsons will assist physicians with optical and mechanical services for developmental work. The Barkan-Koeppe lens is one result of this assistance. The Costenbader Childrens Trial Frame at right is another.

Developmental laboratories, prescription services, the West's most complete surgical and diagnostic showroom.

CLIPS ON TO CHILD'S OWN FRAME. With the Costenbader Childrens Trial Frame the child becomes less disturbed because he wears his own frame during trial examination. Clip the adjustable Childrens Trial Frame on to the front of the frame. Then insert standard size trial lenses.

Write: Dept. 8, 518 Powell St. San Francisco, California

Parsons Optical LABORATORIES

Medical-Journal Report:

Hypertensive symptoms relieved in 96% of patients

"Comparison of pentolinium [ANSOLYSEN] with other preparations in 25 patients with severe essential hypertension . . . showed that pentolinium is . . . most effective . . . in reducing dangerously high blood pressure to the desired levels, and in modifying some of the complications of hypertension, as cardiac decompensation, cardiomegaly and retinopathy. . . .

"In 96 per cent (24 patients) clinical symptoms were relieved and the blood pressure maintained at comfortable levels. . . ."[1]

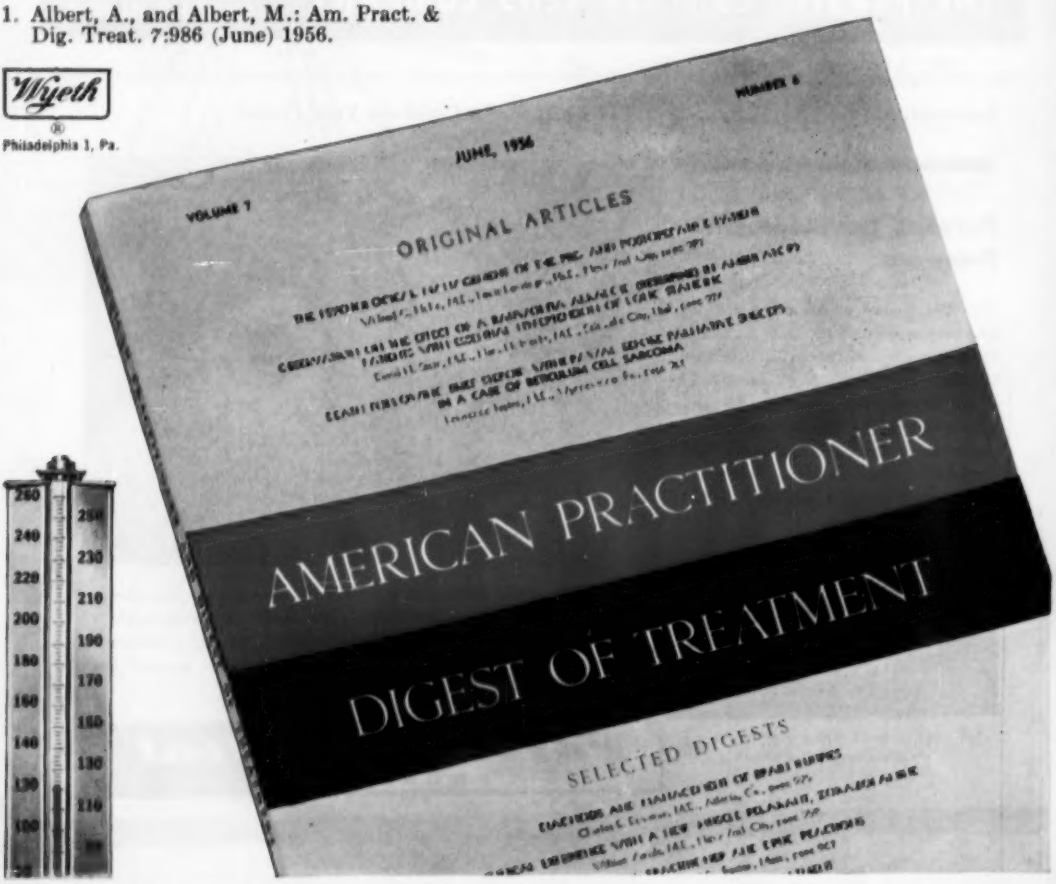
ANSOLYSEN®

TARTRATE — **Pentolinium Tartrate**

Lowers Blood Pressure

1. Albert, A., and Albert, M.: Am. Pract. & Dig. Treat. 7:986 (June) 1956.

Wyeth®
Philadelphia 1, Pa.



Partner in your practice

Dependable,
accurate,
easy-to-use

WELCH ALLYN
Ophthalmoscopes

AO PROJECT-O-CHART

DELUXE FLOOR MODEL
Base matches colors of AO refracting units.

Versatility

for any REFRACTING ROOM

You can enjoy the advantages of subjective testing with the time-saving AO PROJECT-O-CHART and its variety of test slides. If your refracting room presents projection problems because of size or arrangement, there is a Project-O-Chart, in most instances, that can be adapted to your individual needs. Ask your AO Representative for a demonstration. He will be glad to describe the six different models and show you the wide range of test slides available for your use.

STANDARD FLOOR MODEL
Black wrinkle finish, attachment for slide box.

TABLE MODEL
Use on any desk, table or stand.

WALL MODEL
Desirable where floor space is at a premium.

UNIT MODEL
Mounts by bracket on refracting unit.

PORTABLE MODEL
Same as table model with screen and carrying case.

American Optical
INSTRUMENT DIVISION
BUFFALO 15, NEW YORK